DATA EVALUATION RECORD

CARBENDAZIM AND THIOPHANATE-METHYL

STUDY TYPE: EXTENDED ONE-GENERATION REPRODUCTIVE TOXICITY STUDY – RAT (OECD 443-modified)

MRID 49547201

Prepared for
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DATA EVALUATION RECORD

STUDY TYPE: Extended One-Generation Reproductive Toxicity Study; OECD 443 (modified).

PC CODE: 128872 (carbendazim)/102001 (thiophanate-methyl) **DP BARCODE:** D387001

TEST MATERIAL (PURITY): Carbendazim (99.5%)

SYNONYMS: MBC; methyl 1H-benzimidazol-2-ylcarbamate

CITATION: Gilmore, R. (2014) An F_1-extended two-generation reproductive toxicity study with carbendazim in the Wistar rat. Xenometrics, LLC. (Stillwell, Kansas), Laboratory study number 10277, December 23, 2014. MRID 49547201. Unpublished.

SPONSOR: TM/MBC Task Force-MBC Technical Committee (EPA Company No. 83043).

EXECUTIVE SUMMARY: In a modified extended one-generation reproductive toxicity study (EOGRTS) (MRID 49547201), carbendazim (MBC technical, 99.5% a.i.; Batch no. 20080347) was administered to 30 Wistar Hanover Crl:WI(HAN) rats/sex/dose in the diet at dose levels of 0, 250, 1000, or 2000 ppm (equivalent to P generation premating average daily doses of 0, 13.9, 53.2, or 106.7 mg/kg bw/day in males and 0, 16.2, 67.6, and 136.8 mg/kg bw/day in females) for at least 4 weeks prior to mating, during the 14-day mating period, throughout gestation, and through lactation until weaning. Dietary adjustments of dose were not made during the course of any in-life phase of the study. As a result, test compound intake in females during lactation was up to 2-fold greater than other dose groups. The study protocol included evaluation of circulating thyroid hormone levels and a statistical semi-quantitative microscopic evaluation of thyroid colloid area and follicular cell height in parental animals and some offspring cohorts, but did not include an immunotoxicity cohort as recommended in the OECD guidelines. In addition to the mating of P animals, an F1 mating was conducted using Cohort 1a and, separately, Cohort 1b offspring. Details of the methodology used in assessment of thyroid hormones were not provided in the report. Study design and cohort assignment details are summarized below:

P Generation: P males were exposed through postnatal day (PND) 85 and P females were exposed until lactation day (LD) 22. Evaluated parameters at termination included body weight, food consumption, hematology, clinical chemistry, hormone analysis (thyroid and testosterone), organ weights, estrous cycle, ovarian follicle counts, sperm count, motility, and morphology, reproductive performance, gross pathology, histopathology and a semi-quantitative microscopic

analysis of the thyroid follicular cell height and colloid area.

F_1 Generation: Each set of F_1 offspring was maintained on the test diet from the time of weaning until termination. Litter parameters and pup weights were measured until animals were assigned to cohorts at weaning. F_1 offspring were evaluated for potential effects on the nervous system, reproductive and endocrine systems, thyroid function, and other systemic toxicity parameters. In-life parameters evaluated in all F_1 offspring included clinical observations, body weights, food consumption, anogenital distance, nipple retention and puberty onset. Selected F_1 offspring were divided into different groups (Cohorts 1a, 1b, 2a, 2b, and 3) at weaning on PND 21.

Cohort 1a (22/sex/group) and their F_2 offspring: assessment of reproductive and systemic toxicity which included estrous cycle evaluation and post-mortem evaluations that focused on thyroid hormones, reproductive organs, sperm assessment, and ovarian follicle counts. Males and females were mated on PND 90 and males were sacrificed on PND 148. Females were sacrificed on gestation day 20 and cesarean parameters evaluated. The F_2 fetuses from this mating were examined for external, visceral, and skeletal anomalies.

Cohort 1b (20/sex/group) and their F_2 offspring: assessment of reproductive toxicity which included estrous cycle evaluation and post-mortem evaluations that focused on clinical chemistry, hormones (testosterone and thyroid), hematology, reproductive organs, sperm assessment, ovarian follicle counts, and histopathology. Males and females were mated on PND 90 and males were sacrificed on PND 175. Females were sacrificed on LD 20. The F_2 offspring were sacrificed on PND 21 (n=12/sex/group for necropsy, perfusion of the nervous system, and gross brain measurements), PND 23 (n=12/sex/group for thyroid hormone analysis, necropsy and target organ pathology), or PND 45 (20 females/group for vaginal patency and thyroid hormone analysis).

Cohort 2a (10/sex/group): developmental neurotoxicity assessment, which included clinical signs, body weight, ophthalmology (~PND 45), functional observational battery (FOB, PND 52-55), motor activity (PND 63-66), and acoustic startle response (PND 58-61). On PND 70, animals were perfused for central nervous system and peripheral nerve neuropathology evaluation and brain morphometry.

Cohort 2b (12/sex/group): developmental neurotoxicity assessment, which included clinical signs, terminal body weight and brain measurements, perfusion for central nervous system and peripheral nerve neuropathology evaluation, and brain morphometry on PND 21.

Cohort 3 (12/sex/group): assessment of systemic toxicity which included thyroid hormone analysis, necropsy, and target organ pathology on PND 23.

Parental toxicity (P and F_1 adult animals): There were no treatment-related effects on P or F_1 parental mortality, clinical signs, body weight, body weight gain, food consumption, hematology or clinical chemistry parameters, urinalysis and macroscopic findings. At 2000 ppm, P males showed decreases in monocytes (counts -29% and percent -33%) but values were within historical control range. In F_1 Cohort 1b, significantly increased white blood cell counts (40%) and absolute lymphocytes (46%) and segmented neutrophils (35%) were observed in parental females. These changes were not considered adverse because they were within historical control range or, for segmented neutrophils, most or all groups (including concurrent controls) exhibited counts greater than the historical controls. Significantly decreased triglyceride levels in P females at

2000 ppm and F_1 parental females at 1000 and 2000 ppm were observed but are considered-non adverse as no gross or histological correlates were observed, and decreases in triglyceride levels are typically not an adverse effect.

Plasma testosterone levels in P males were minimally reduced at 1000 and 2000 ppm (25% and 24%, respectively; not statistically significant) but there was significant variation among individual animals and there were no effects on male sperm or reproductive parameters. In P males at 2000 ppm, very slight decreases in testes weight (4% below controls) and minimal or slight testicular atrophy (3/30) were not considered adverse due to the marginal change and lack of sperm and reproductive effects.

Changes in thyroid parameters were seen in P and adult F_1 animals but were most pronounced in P females, with alterations in both plasma hormone levels and thyroid histopathology observed at 1000 and 2000 ppm. In P females, plasma T4 levels were increased in all treatment groups (27, 34 and 18%, $p>0.05$ at 250 and 1000 ppm), as were TSH levels (64, 164 and 92%, $p<0.05$ at 1000 ppm). Minimal to slight microscopic follicular cell hypertrophy was observed at 1000 (2/29) and 2000 (3/23) ppm in P females only. A statistically significant decrease in colloid area and increase in follicular cell height was observed in the semi-quantitative microscopic analysis of the thyroid at 1000 and 2000 ppm. No thyroid effects were observed in P males except for a nonsignificant decrease (19%) in T4 at 2000 ppm.

In adult F_1 offspring, no thyroid effects were observed in Cohort 1a males (PND 148) or females (GD 20). F_1 Cohort 1b males (PND 175) showed a statistically significant decrease in T3 (19% and 18%) at 1000 and 2000 ppm, but no other effects on thyroid hormones or histopathology. Testosterone levels were also unaffected by treatment. F_1 Cohort 1b females (LD 22) showed no effects on thyroid hormones, but at 2000 ppm, significant increases in absolute/relative thyroid weight (18%/25%). Decreased colloid area/increased follicular cell height were also observed. Analysis of follicular cell height and T4 or TSH levels did not show a consistent positive correlation among the different cohorts/generations of treated animals. However, in P females at LD 22, the combined findings of changes in T4 and TSH, along with slight thyroid hypertrophy and increased follicular cell height/decreased follicular colloid area seen at 1000 and 2000 ppm, were considered indicative of perturbation of thyroid homeostatis, and therefore an adverse effect. **The parental toxicity LOAEL is 1000 ppm (53.2 mg/kg bw/day, males and 67.6 mg/kg bw/day in females, based on increased thyroid hormones and histopathological changes in P females during lactation (LD 22). The NOAEL for parental toxicity is 250 ppm (premating intake of 13.9 mg/kg bw/day, males and 16.2 mg/kg bw/day, females).**

Reproductive toxicity: There were no treatment-related effects on reproductive indices, precoital intervals, gestation length, sexual development of F_1 parental animals, estrous cycle, ovarian follicle count, sperm parameters [number, motility, and morphology], parturition, lactation, or tissues and organs of the reproductive system. **The reproductive NOAEL is 2000 ppm (106.7 mg/kg bw/day in males, 136.8 mg/kg bw/day in females), the highest dose tested. The LOAEL for reproductive toxicity is not established.**

Developmental toxicity (prenatal only): Fetuses from the F_1 Cohort 1a group mating were evaluated on GD 20. There were no treatment-related effects on live litter size, fetal anomalies (external, visceral or skeletal), fetal weight, or fetal sex ratio. **The developmental NOAEL is 2000 ppm (106.7 mg/kg bw/day in males, 136.8 mg/kg bw/day in females), the highest dose tested. The LOAEL for developmental toxicity is not established.**

Offspring toxicity (systemic toxicity and developmental landmarks): There were no treatment-related effects on live litter size or pup viability, anogenital distance, developmental landmarks, food consumption, organ weights, or histopathology of the F_1 and F_2 offspring. The number of litters with pups found dead was increased in the P high-dose group (6/27, 22%) compared with controls (1/30, 3%) and the number of F_1 pups found dead during the lactation period was 15 and 1 for the high-dose and control group, respectively. However, the increased post-natal death was considered incidental since the majority (10) of the pups were from one litter with complete loss during the lactation period and an increase in dead pups was not seen in treated F_2 offspring. Body weight gain of F_1 male pups at 2000 ppm was significantly decreased from PND 4-18 by 10%, correlating with absolute mean body weights decreases of 6-7% (n.s.) from PNDs 14-21. After weaning, body weights of F_1 male offspring in both Cohorts 1a and 1b remained decreased, compared with controls, from PNDs 24-28 by 7-10% (s.s.), but were not significantly decreased at later time points. Mean body weights of F_2 females were significantly decreased by 8-11% on PNDs 24-28. The decreased body weights in offspring was transient but considered adverse due to the young age of the animals.

Changes in thyroid hormone levels, colloid area and follicular cell height were seen in offspring but were not consistent across cohorts or life stages. In F_1 PND 4 offspring, decreased T4 was observed at 1000 and 2000 ppm (24% and 35%; significant at high dose) but TSH was unaffected (histopathology was not evaluated). At PND 23, male and female F_1 pups (cohort 3) at 2000 ppm showed statistically significant increases in T4 (53%/22%), significant increases in TSH in females (41%) and significantly decreased colloid area/increased follicular cell height in both sexes (males -15%/+55%; females -22%/+85%). PND 23 males at 1000 ppm also showed significant increases in T4 and TSH (42%/21%) and significant colloid area/follicular cell height changes (-12%/+43%); in females, only TSH was increased (28%, n.s.) at this dose level. In contrast, F_2 pups at PND 23 showed only nonsignificant increases in T4 in males (20%) and TSH in females (26%). PND 45 females at 2000 ppm showed increased T4 (52%, $p<0.05$), TSH (41%, n.s.) and significantly altered colloid area and follicular cell height (-19%/+60%). Analysis of the correlation between follicular cell height and T4 or TSH levels did not show a consistent positive correlation in treated animals; however, the hormone changes were considered adverse to be protective of potential hormone changes during development. No changes in thyroid parameters were observed in any offspring at 250 ppm. **The offspring toxicity LOAEL is 1000 ppm (53.2 mg/kg bw/day in males, 67.6 mg/kg bw/day in females), based on thyroid hormone and histopathology alterations in F_1 PND 23 male pups and decreased T4 in F_1 PND 4 pups (pooled litter blood). The offspring toxicity NOAEL is 250 ppm (13.9 mg/kg bw/day in males, 16.2 mg/kg bw/day in females).**

Offspring developmental neurotoxicity: There was no effect of treatment on clinical signs, ophthalmology, FOB parameters, motor and locomotor activity, auditory startle parameters, brain weights, gross brain measurements, microscopic brain measurements and brain neuropathology, or other neuropathological findings. Statistically significant differences in brain morphometric measurements were of small magnitude and did not show a dose-response and were therefore not considered treatment-related. **The developmental neurotoxicity NOAEL is 2000 ppm (106.7 mg/kg bw/day in males, 136.8 mg/kg bw/day in females), the highest dose tested. The LOAEL for developmental neurotoxicity is not established.**

This study is classified as **Acceptable / Non-Guideline** and does not fully satisfy the guideline requirement for an extended one-generation reproductive toxicity study (OECD 443) in the rat. The guideline was modified to test the potential reproductive, developmental neurotoxicity, and

thyroid effects of the test substance and is adequate to assess these parameters, but it is noted that detailed SOPs for thyroid hormone analysis, conducted at a separate laboratory, were not available. The immunotoxicity evaluations, recommended in OECD TG 443 for Cohort 3 animals, were not conducted. However, the study, as conducted, does not show evidence of immunotoxic potential of carbendazim, based on lack of observed effects on hematology, organ weight or histopathology.

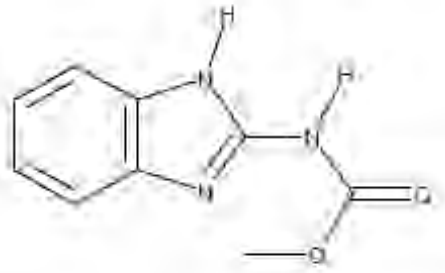
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Carbendazim

Description:	White powder
Batch #:	20080347
Purity:	99.5% a.i.
Compound stability:	Stable at room temperature; protected from light
CAS # of TGAI:	10605-21-7
Structure:	



2. Vehicle and/or positive control: Acetone diluent for dietary premix; mixed in Purina Mill Certified Rodent Diet 5002 in "meal" form.

3. Test animals:

Species:	Rat	
Strain:	Wistar Hanover Crl:WI (HAN)	
Age at study initiation:	11 weeks	
Wt. at study initiation:	P males: 282.0-373.3 g; P females: 181.9-222.6 g F_1 males: 45.6-48.6 g; F_1 females: 43.8-45.6 g	
Source:	Charles River Laboratories, Inc., Raleigh, NC	
Housing:	Singly housed in suspended stainless-steel wire mesh cages, except during mating; gestation and lactation- dams and litters housed in polycarbonate cages with heat treated Aspen shavings bedding; PND 21-27: F_1 and F_2 pups housed by litter (separated by sex) in polycarbonate cages; PND 28-sacrifice: F_1 and F_2 pups individually housed in stainless-steel wire mesh cages	
Diet:	Purina Mills Certified Rodent Diet 5002, *ad libitum*	
Water:	Deionized water, *ad libitum*	
Environmental conditions:	**Temperature:**	18-26°C
	Humidity:	30-70%
	Air changes:	11.58/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	7 days	

B. PROCEDURES AND STUDY DESIGN

1. In life dates: Start: May 14, 2012. Dosing initiation May 29, 2012; End: January 17, 2013

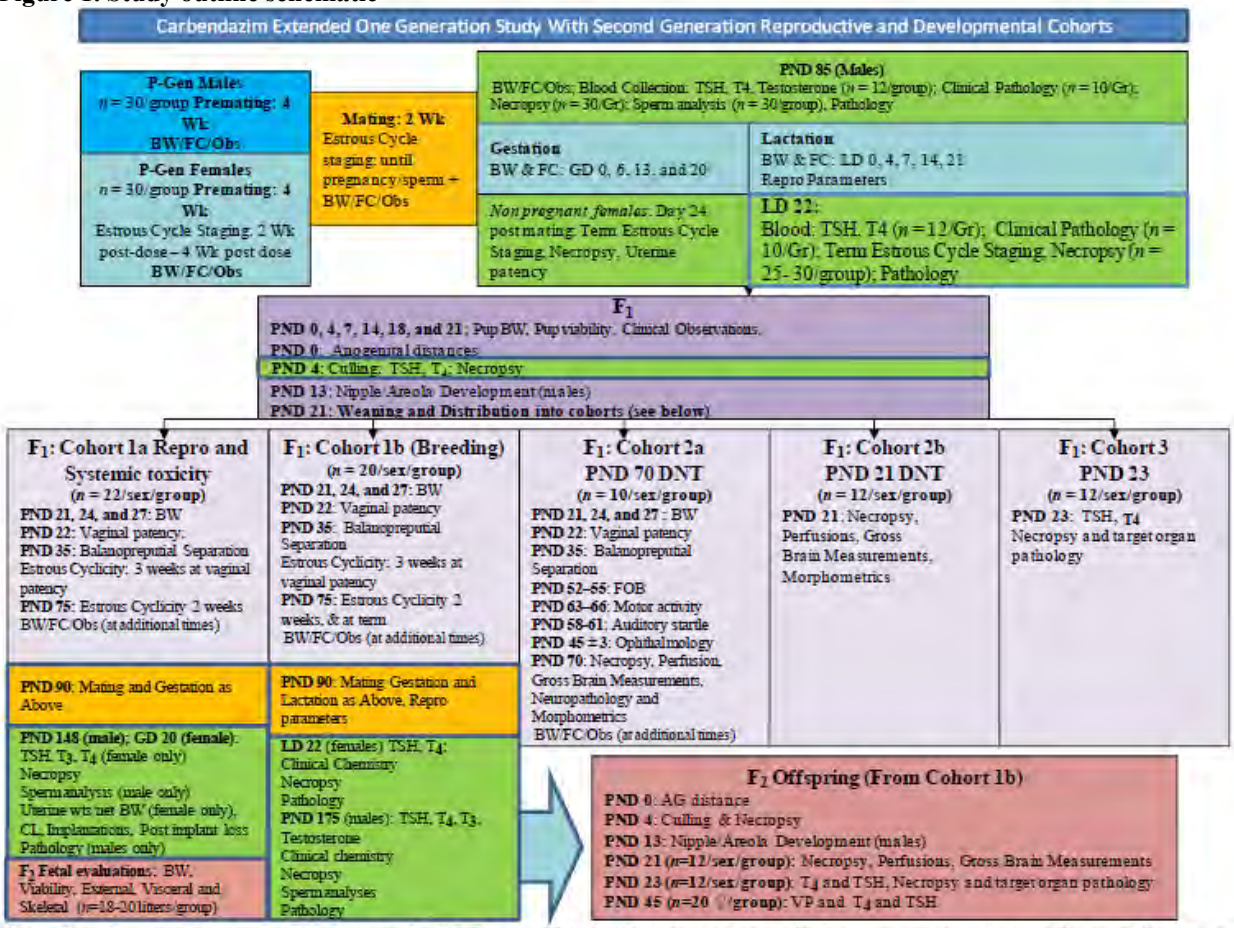
2. Mating procedure: Each female was housed with a male from the same test group for up to 14 days. Mating for F_1 Cohorts 1a and 1b animals began on postnatal day (PND) 90, and sibling mating was avoided. Vaginal smears were taken daily and examined for the presence of sperm or copulatory plugs. The day on which sperm or a copulatory plug was observed was designated gestation day (GD) 0, and females were removed from the males and placed in nesting cages. After the mating period, females with no evidence of mating were separated from the males and placed in nesting cages.

3. Study schedule: A schematic of the study outline is shown in Figure 1, obtained from the study report. P parental animals received the test diet for 4 weeks prior to mating, during the mating period (14 days), throughout gestation and lactation, and up until sacrifice. P parental males were sacrificed on PND 85 and P parental females were sacrificed on lactation day (LD) 22. The F_1 generation was weaned on PND 21. Females that had evidence of mating but did not deliver or did not have evidence of mating were sacrificed on GD 24 or at least 24 days after the last day of the mating interval. On PND 4, the size of each litter was adjusted, when possible, to 5 males and 5 females per litter, with extra pups sacrificed. Pups from each litter were selected randomly for assignment to the adjusted litters using SAS software. Litters with fewer than 5 males or 5 females were partially adjusted so that a total of ten pups were available per litter, and litters with fewer than ten pups were not adjusted. At weaning on PND 21, F_1 offspring were divided into five Cohorts as follows:

Cohort 1a- reproductive and systemic toxicity,
Cohort 1b- reproductive toxicity with breeding,
Cohort 2a- adult developmental neurotoxicity,
Cohort 2b- pup developmental neurotoxicity, and
Cohort 3 necropsy with target organ pathology.

Cohort 1a males received the test diet until sacrifice on PND 148, and females received the test diet until sacrifice on GD 20 for cesarean section evaluation and fetal examination. Cohort 1b animals produced the F_2 generation which was culled on PND 4 and sacrificed on PNDs 21, 23, and 45. Cohort 1b parental males were sacrificed on PND 175 and parental females were sacrificed on LD 22. Cohort 2a animals received the test diet until sacrifice on PND 70. Cohort 2b animals were sacrificed on PND 21. Cohort 3 animals were sacrificed on PND 23. Offspring in both generations not selected for a Cohort or culled were sacrificed on PND 4.

Figure 1. Study outline schematic[a]



Note: This schematic above is to provide major study-related events. Not all individual activities are listed; see appropriate section(s) of the report for more specific details related to each activity).

[a] Taken from p. 21, MRID 49547201.

4. **Animal assignment:** Animal assignment is given in Table 1. Animals were randomly assigned to a group using a weight stratification-based computer program such that the body weights fell within ±20% of the mean (per sex) for all animals. Assignment of F_1 offspring was determined by birth order of the litters. The F_1 and F_2 offspring were assigned using the SAS PND 4 randomization printouts. Priority was given to equal litter representation for Cohort 2a followed by Cohort 1b, Cohort 2b, and Cohort 1a to avoid representation by more than one pup/litter.

TABLE 1. Study design [a]

Dose (ppm)	Group	Male	Female
0	P parental animals	30/dose	30/dose
250	F_1 Cohort 1a	22/dose	22/dose
1000	F_1 Cohort 1b	20/dose	20/dose
2000	F_1 Cohort 2a	10/dose	10/dose
	F_1 Cohort 2b	12/dose	12/dose
	F_1 Cohort 3	12/dose	12/dose
	Cohort 1b F2 offspring	12/dose	12/dose for PND 23 (20/dose for PND 45)

[a] Taken from pp. 37-46, MRID 49547201.

5. **Dose selection rationale:** The dose levels were selected after consultation with the US EPA and the Canadian PMRA and based on the results from a reproductive toxicity dose range-

finding study with toxicokinetics in which Wistar rats were fed diets containing the test substance at doses of 300, 1000, 3000, and 6000 ppm (MRID 48277601). During lactation and the post-weaning period, the doses were decreased by 50% to maintain a more constant mg/kg bw/day exposure. Minimal toxicity was observed in adult animals at 6000 ppm: slightly decreased maternal body weight during late gestation and mild sperm and testicular effects were observed. Decreased body weights of pups was observed at 3000 and 6000 ppm. A slight decrease in litter size was observed at 3000 and 6000 ppm and may have been related to exposure. One pup died after weaning in the 3000 ppm group and one pup died and another was sacrificed *in extremis* in the 6000 ppm group. Toxicokinetics results showed that the test substance and its metabolite were bioavailable from the diet and transferred through lactation to the offspring. The doses in the current study were set at 250, 1000, and 2000 ppm and were not decreased during lactation or post-weaning.

6. **Test material intake:** The average daily intake for P and F_1 animal groups at each dietary concentration is shown below in Table 2. It is noted that the average daily intakes were up to two-fold higher in lactating P and F_1 females than other groups since food consumption per kg bw is greater during lactation and test diet concentrations were not adjusted.

TABLE 2. Mean test substance intake (mg/kg bw/day)[a]

Group	Dose (ppm)		
	250	1000	2000
P parental males	13.9	53.2	106.7
P parental females- premating	16.2	67.6	136.8
P parental females- gestation	17.9	74.2	146.6
P parental females- lactation[b]	32.4	135.8	258.0
F_1 Cohort 1a males	16.9	67.0	142.6
F_1 Cohort 1a females-premating	20.9	87.9	173.4
F_1 Cohort 1a females-gestation	18.0	74.3	146.7
F_1 Cohort 1b males	16.6	66.2	136.9
F_1 Cohort 1b females-premating	20.9	90.8	180.5
F_1 Cohort 1b females-gestation	18.2	76.2	148.0
F_1 Cohort 1b females-lactation[b]	33.6	124.6	264.3
F_1 Cohort 2a males	23.9	90.2	197.9
F_1 Cohort 2a females	26.1	99.2	237.2

[a] Data obtained from Text Table 16, p. 75, MRID 49547201.

[b] Mean includes LD 0–14; LD 14-21 excluded from calculation due to confounding data as pups also ate feed during this time.

7. **Dosage preparation and analysis:** The test diet formulations were corrected for test substance purity. The test substance was dissolved in acetone (evaporated off prior to use) and mixed with the diet at room temperature. The test diet formulations were prepared weekly and stored at -20 ± 5°C until the subsequent week when it was given to the animals. Stability (freezer temperature at 0, 36, and 49 days) was determined prior to initiation of exposure at doses of 100 and 3100 ppm. Homogeneity was determined in the reproductive toxicity dose range-finding study (MRID 48277601) and data were not provided in this study report. Concentration of the test substance in the diet was determined prior to initiation of treatment and at monthly intervals. Concentration was also determined from samples at the beginning of gestation and lactation of each generation.

   In addition, the basal test diet (Purina Mills Certified Rodent Diet 5002) was assayed for levels of isoflavones (phytoestrogens) that could affect the results of estrogen-dependent

parameters in the study. The analysis was undertaken because the study was re-initiated using the Purina diet, due to pup death resulting from clogging of the nares by the low-phytoestogen diet (Harlan Teklad® Certified Global 16% Protein Rodent Meal 2016 CM) that was originally selected for the study. Analyses were performed on 3 dates for 3 batches used in the study (Table 3). Although the total isoflavone levels were above 500 aglycone equivalents, there were no treatment-related findings related to estrogen-dependent parameters in this study; therefore, the diet selection was considered adequate.

Table 3. Results of isoflavone profile analysis of Purina Certified Rodent Diet 5002 used in the study[a]

Isoflavone Profile Parameter	Diet Lot Results (ppm)			Overall Mean of All Diet Lots in ppm
	April 03 12 2B	Jun 01 12 3B	October 17 12 2A	
Total Daidzein (Aglycone Units)	224	231	203	219
Total Genistein (Aglycone Units)	276	274	215	255
Total Glycitein (Aglycone Units)	38	27	28	31
Total Isoflavones (Aglycone Equiv)	**538**	**532**	**446**	**505**

[a] Data extracted from Text Table 17, p. 75 of MRID 49547201.

Results:

Stability analysis: The test substance was found to be stable when mixed with the diet at 100 and 3100 ppm after storage in the freezer. For the 100 ppm sample, the mean measured concentrations were 96.7, 108, and 99.5 ppm at 0 days, 36 days, and 49 days after preparation, respectively. The mean measured concentrations of the 3100 ppm sample were 3002, 3118, and 2988 ppm at 0 days, 36 days, and 49 days after preparation, respectively. The percent differences from day 0 at 36 and 49 days were 11.6% and 2.8%, respectively, at 100 ppm. At 3100 ppm, the percent differences from day 0 at 36 and 49 days were 3.9% and -0.5%, respectively.

Concentration analysis: No test substance was detected in the control samples. The mean measured concentrations of the test substance were generally within ±15% of nominal. On one occasion, the results for all dose formulations were > 18% of target. On a different occasion, the high-dose formulation was 16.9% of target.

The analytical data indicate that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

8. **Dosage administration:** The test diets were available to the animals *ad libitum*.

C. OBSERVATIONS:

1. **Parental animals:** Observations and the schedule for those observations are shown in Figure 1 (reproduced from study report page 21).

   a. **Mortality and clinical observations:** Animals were observed twice daily for mortality, morbidity, behavioral changes, signs of difficult or prolonged delivery, and overt toxicity. Detailed clinical examinations were performed weekly. When possible clinical observations were observed during the cage-side-evaluation, the animal was removed from the cage and a detailed assessment conducted. A detailed evaluation of clinical observations including observing the animal in the cage and removing the animal to perform a physical examination was conducted at least once per week (on a day animals were weighed) throughout the entire in-life phase of the study.

b. Body weight: Parental males were weighed on the first day of treatment, and weekly throughout the study. Parental females were weighed on the first day of treatment, weekly during premating, on GDs 0, 6, 13, and 20, and on LDs 0, 4, 7, 14, and 21.

c. Food consumption: Food consumption of parental males was measured weekly during pre- and post-mating. Food consumption of parental females was measured at the same interval as body weight except during the first week of lactation. Food consumption was measured from LDs 0-4 and 4-7 during the first week. Test substance intake was calculated using food consumption, dietary concentrations, and body weight measurements.

d. Estrous cycle: Vaginal smears to determine estrous cycle length and pattern were collected daily from parental females for two weeks during pre-mating and during mating until conception was confirmed.

2. Litter observations: F_1 and F_2 pup observations are shown (X) in Table 4. Anogenital distance was measured in all F_1 and F_2 pups on PND 0. On PND 13, nipple/areola retention was evaluated in all male F_1 and F_2 pups.

TABLE 4. Litter observations [a]

Observation	Time of observation (postnatal day)					
	PND 0	PND 4	PND 7	PND 14	PND 18	PND 21
Number and sex of liveborn pups	X					
Number and sex of stillborn pups	X					
Clinical observations	Once daily					
Pup weight (individual)	X	X	X	X	X	X
Gross anomalies	X					

[a] Data obtained from pp. 61-62, MRID 49547201.

On PND 4, litters were standardized to a maximum of 10 pups/litter (5/sex/litter, as nearly as possible). Software from SAS was used to randomly select which F_1 and F_2 generation pups would continue on study.

All pups found dead were examined for gross lesions and for the cause of death. [See below.]

3. F_1 offspring:

a. Mortality and clinical observations: Animals were observed twice daily for mortality, morbidity, behavioral changes, signs of difficult or prolonged delivery, and overt toxicity. Detailed clinical examinations were performed weekly. Cohort 1a and 1b dams were given detailed examinations for clinical observations on GDs 0, 6, 12, 18, and 20.

b. Body weight: Animals were weighed on PNDs 21, 24, and 27 and then weekly until sacrifice. Cohort 1a dams were weighed on GD 0, 6, 9, 12, 15, 18, and 20. Cohort 1b dams were weighed on GDs 0, 6, 13, and 20 and LDs 0, 4, 7, 14, and 21. Cohort 2a offspring were also weighed on day of FOB and acoustic startle measurement.

c. Food consumption: Food consumption was measured weekly and during the same interval as weight measurements for the Cohort 1a and 1b dams. Test material intake was calculated using food concentrations, body weights, and food consumption data.

d. **Estrous cycle:** Vaginal smears were collected from Cohort 1a and 1b females for three weeks beginning on the day of vaginal opening, and then on PND 75 for an additional two weeks. The stage of the estrous cycle was also determined for all Cohort 1b females at time of necropsy.

e. **Anogenital distance:** The distance from the center of the anal opening to the base of the genital tubercle, or anogenital distance (AGD), was measured for all F_1 and F_2 male and female pups on PND 0. Body weight was measured on the same day the AGD measurements were taken.

f. **Nipple retention:** All surviving F_1 and F_2 Cohort 1b offspring male pups were examined on PND 13 for the presence or absence of nipple/areola anlagen.

g. **Sexual maturity:** F_1 Cohorts 1a, 1b, and 2a males and females and F_2 females were evaluated daily post-weaning for balanopreputial separation (from PND 35) or vaginal opening (from PND 22). The age and weights were recorded when animals attained complete balanopreputial separation and vaginal opening.

h. **F_1 Cohort 2a ophthalmology and developmental neurotoxicity:** On ~PND 45, ophthalmologic examination was conducted. A Finnoff transilluminator was used to examine the anterior portions of the eyes, and the retina was examined using an indirect ophthalmoscope following dilation with a mydriatic agent.

The 10 rats/sex/group designated for neurobehavioral assessments were subjected to FOB testing between PND 52 and 55, motor activity testing once on PND 63-66, and auditory startle on PND 58-61. Animals were transferred to the room where testing took place and allowed to acclimate with minimal disturbance for at least 30 minutes before testing. The FOB included home cage, open field, manipulative, and physiologic observations in a standard animal room on the same light/dark cycle. The same observer was used throughout the study and was unaware of the group assignment. The same or a different observer was used for grip strength and foot splay measurements. Studies were previously conducted with acrylamide, carbaryl, and untreated rats to establish the sensitivity, reliability, and validity of the test procedures and to serve as a historical control (Sheets, P.P., Historical Control and Method Validation Studies in Rats for the Acute and Subchronic Neurotoxicity Screening Battery, Bayer Crop Science LP (formerly Miles, Inc.) LP Report No. 103979, MRID 42779391, 1993).

The methods, environmental conditions, duration of observations, strain gauges, and scoring criteria were not adequately described within the study report; references for established procedures were cited. The animals were tested individually for 60 minutes in 8 figure-8 mazes for motor and locomotor activity. Activity counts were measured by beam interruption (8 infrared emitter-detector pairs were present in the mazes) for the 60-minute sessions as well as activity during each 10-minute interval. A Columbus Instruments Universal Maze Monitoring system and personal computer were used for automated data collection during each session. Motor activity was measured as the number of beam interruptions, and locomotor activity was measured by eliminating consecutive counts for a given beam. For locomotor activity, only one interruption of a given beam was counted until the rat relocated in the maze and interrupted another beam. Habituation was evaluated as a decrement in activity during the test session.

Auditory startle reflex habituation testing was performed using a computer-controlled automated system (Coulbourn Acoustic Startle, Version 3.2.10-00; Coulbourn Instruments) in which the animals were presented with the startle-eliciting stimulus for 10-second intervals during 50 trials. Up to 4 animals per group were tested simultaneously in each of two startle system enclosures. The enclosures were ventilated and lined with sound-damping and vibration-absorbing material with a ceiling-mounted speaker providing a 50 msec, 118 dB stimulus (white noise). The enclosures also contained transducer assemblies to measure startle response. Animals were placed in individual restraining cages on top of each load cell and were given a 5-minute adaption period at ambient noise levels prior to initiation of the trials. Response was recorded for 200 msec after stimulus presentation and measured as the force (maximum value of the average curve) exerted on a platform minus baseline (animal weight, based on the average force in grams exerted on the platform during the first 8 milliseconds of the stimulus. For each animal, peak response amplitude in grams and latency in msec were measured. Latency to peak was measured as the time in msec after the stimulus onset to the time of peak response amplitude. The average response amplitude and habituation (magnitude of decrease) in blocks of 10 trials were compared for each dose group.

The CHECKED (X) parameters were examined:

	HOME CAGE OBSERVATIONS		HANDLING OBSERVATIONS		OPEN FIELD OBSERVATIONS
X	Posture	X	Reactivity	X	Rearing count
	Biting	X	Lacrimation/ chromodacryorrhea	X	Arousal/ general activity level
X	Convulsions	X	Salivation	X	Convulsions
	Tremors	X	Piloerection		Tremors
	Abnormal Movements	X	Fur appearance		Abnormal movements
X	Palpebral closure	X	Palpebral closure	X	Urination / defecation
	Feces consistency		Eye prominence		Grooming
X	Bizarre / stereotypic behavior	X	Respiration characterization	X	Gait abnormalities / posture
X	Reaction to removal		Red/crusty deposits*	X	Bizarre / stereotypic behavior
	SENSORY OBSERVATIONS		Mucous membranes /eye /skin color		Backing
					Time to first step
X	Visual reaction	X	Muscle tone	X	Vocalization
X	Touch response		Extensor thrust		
X	Auditory response				
X	Pain (tail pinch) response		**PHYSIOLOGICAL OBSERVATIONS**		
	Visual placing response				
X	Air righting reflex	X	Body weight		
	Pupil size and response	X	Body temperature		**NEUROMUSCULAR OBSERVATIONS**
X	Startle response	X	Pupil response		
	Eyeblink response	X	Pupil size		Hindlimb extensor strength
	Forelimb extension		**OTHER OBSERVATIONS**	X	Forelimb grip strength
	Hindlimb extension	X	Motor activity	X	Hindlimb grip strength
	Olfactory orientation	X	Locomotor activity	X	Landing foot splay
	Approach response				Rotarod performance

Taken from Appendix C (p. 1357), MRID 49547201.

4. **<u>Hematology and clinical chemistry</u>:** On the scheduled day of necropsy, blood for hematology and clinical chemistry parameters was collected from the orbital sinus of fasted P parental and F_1 Cohort 1b animals (10/sex/group, excluding those used for thyroid hormone

analysis) under isoflurane anesthesia. The included animals were the second 10 adult males and lactating females. The CHECKED (X) parameters were examined (taken from page 50 of the report).

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
X	RBC morphology	X	Red cell volume distribution width
	Blood clotting measurements*	X	Hemoglobin distribution width
	Prothrombin time (PT)		
	Activated partial thromboplastin time (APPT)		

* Recommended for studies based on OECD 443.

b. Clinical chemistry (taken from page 53 of report)**:**

X	ELECTROLYTES	X	OTHER
X	Calcium	X	Albumin*
	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total cholesterol*
	Potassium*	X	Globulins
	Sodium*	X	Glucose*
	ENZYMES (at least 2 hepatic enzymes*)	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
X	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		Bile acids
X	Alanine aminotransferase (ALT/also SGPT)*	X	Albumin/globulin ratio
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
	Gamma glutamyl transpeptidase*		
	Glutamate dehydrogenase		

* Recommended for studies based on OECD 443.

5. Urinalysis: Urine was collected overnight from fasted P parental and F_1 Cohort 1b animals (10/sex/group) housed in cages fitted with urine collection trays. The CHECKED (X) parameters were examined (taken from page 54 of report).

X	Color	X	Bilirubin
X	Appearance*	X	Glucose*
X	Specific gravity*	X	Protein*
X	Volume*	X	Ketones
X	pH*	X	Blood/blood cells*
X	Urobilinogen	X	Sediment*
X	Nitrites	X	Leucocytes

* Recommended for studies based on OECD 443.

6. Hormone analysis: The report did not state whether animals were kept in a holding room prior to sacrifice to minimize stress but this was confirmed via personal communication (A. Krygsman to J. Hardy and L. Hansen, email dated 1/22/18). After a short time (less than 2

minutes) under isoflurane anesthesia, blood (non-fasting) for thyroid hormone and/or testosterone analyses was collected following decapitation of the first 12 adults/group as follows: P (PND 85) and F_1 Cohort 1b (PND 175) males, P and F_1 Cohort 1b females on LD 22 after weaning, and from F_1 Cohort 1a females on GD 20. Blood was also collected from 12 male and females/group F_1 and F_2 pups on PND 4 and 23 (F_1 Cohort 3) and from 12 female F_2 pups on PND 45. PND4 pups from both generations were also sacrificed via decapitation and blood pooled within litters for potential thyroid hormone analysis; however, hormone analyses were only performed for the group F_1 pups.

Blood samples were collected into tubes without additive (-approximately 600 ul for testosterone assays and -1000 ul for thyroid assays). Deviations to the procedures were noted on page 52 of the study report. Blood was allowed to clot at room temperature for at least 30 minutes prior to centrifugation. Blood sampling for either thyroid hormone or sex hormone analyses were balanced across groups and completed within a three or four hour window in the morning of the day of necropsy (noted that 8 P-generation males were bled outside the three hour window by 25 and 27 minutes, respectively. See page 52 of study report). Blood from PND4 pups was only analyzed for thyroid hormones. All blood samples were transferred to the clinical pathology department as soon as possible after collection.

Thyroid hormone analysis was performed by Ani Lytics, Inc. using a method developed in-house (SOP numbers 6-4A and 6-11). The SOPs were not provided in the study report, but limited information was provided via personal communication (A. Krygsman to J. Hardy, email dated 11/13/2017. The rat TSH assay is an in-house RIA using a rat-specific TSH cold standard, iodination standard and primary antibody provided by the National Hormone and Peptide Program, CA. The precipitating antibody is goat anti-rabbit IgG (MP Biomedical, OH). For T3 and T4 assays, solid phase coated tube RIAs were used (MP Biomedical). Ani Lytics, Inc. is no longer in business and no further details were available.

X	Thyroxine (T4)*	X	Triiodothyronine (T3)#
X	Thyroid stimulating hormone (TSH)*	X	Testosterone

* Recommended for studies based on OECD 443.
\# F_1 cohorts 1a females and 1b males (T^3 not analyzed for cohort 1b females.)

7. <u>Postmortem observations and reproductive toxicity</u>:

a. <u>P parental and F_1 parental Cohort 1a and 1b animals</u>: With the exception of the animals that provided blood for hormone analyses (see above), males and females were euthanized by carbon dioxide asphyxiation. P parental males were sacrificed on PND 85 and parental females were sacrificed on LD 22. F_1 Cohort 1a parental males were sacrificed on PND 148 and parental females were sacrificed on GD 20. F_1 Cohort 1b parental males were sacrificed on PND 175 and parental females were sacrificed on LD 22. All animals were subjected to a full, detailed gross necropsy with special attention paid to the reproductive organs (see table below). Females that had evidence of mating but did not deliver or did not have evidence of mating were sacrificed on GD 24 or at least 24 days after the last day of the mating interval and subjected to gross necropsy. The uteri of these females were flushed with 10% buffered formalin to determine cervical/uterine *os* patency, and if patent, were examined for possible implantation sites. The uterus and ovaries of F_1 Cohort 1a females who did not become pregnant were fixed in 10% buffered formalin and retained for possible histopathology.

Female reproductive parameters: Implantation sites of all pregnant dams were counted using a dissecting scope and the stage of estrous was determined. Quantitative evaluation of the ovarian preantral and antral follicles and the corpora lutea was conducted on 20 females in the control and high-dose groups of the P parental generation and in F_1 Cohort 1b (based on 5 sections taken from the left ovary). Low and mid doses were not examined because the high dose values were within 10% of controls, the normal variability for this measure. In F_1 Cohort 1a females, gravid uterus weights were recorded and corpora lutea were counted. All resorptions were characterized and placentas were trimmed and weighed after removal of the fetuses from the uterine wall.

Male reproductive parameters: The testes and epididymides from all dose groups were examined. The vas deferens of the controls and high-dose group males from the P and F_1 Cohort 1b generation and all males from F_1 Cohort 1a males were examined. Histopathology was performed on the reproductive organs of all animals (except F_1 Cohort 1a females) suspected of reduced fertility and those with abnormal estrous cyclicity or sperm parameters. In all P parental and F_1 Cohort 1a and 1b males, a stage-dependent qualitative histopathology assessment was conducted on the right testes and epididymis. Normal stage progression, spermatogenic cycle, and cell association and proportions, were determined. Sperm was collected from one testis and epididymis from all P parental males, and the first 10 males/group of F_1 Cohort 1a and 1b males for enumeration of homogenization-resistant spermatids and cauda epididymal sperm reserves, respectively. Sperm count, morphology (control and high-dose groups) and motility was examined in samples collected from the distal portion of the vas deferens.

Thyroid: Histopathological examination of thyroids was conducted using the Tier 1 EDSP Pubertal protocol (OPPTS 890.1450): Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Female Rats). This guidance indicates a subjective evaluation of follicular cell height and colloid area using a five point grading scale where 1 = shortest/smallest and 5 = tallest/largest. The follicular cell height and colloid area of the thyroid glands in P parental males and females, F_1 Cohort 1a males, and F_1 Cohort 1b males and females were scored using this system. At least two sections of two lobes of the thyroid were evaluated using the 5-point grading scale. Each section was given a score and the mean score/animal was calculated.

The table below (data extracted from study report text Table 14, pp. 56-57) summarizes the organs or tissues that were collected from all dose groups. The indicated (X) organs or tissues were preserved in appropriate fixative, and selected (XX) organs, in addition, were weighed. Histopathology was performed for the first 10-12 control and high-dose group animals from the P parental animals and F_1 Cohort 1b animals.

Organs examined	P generation	F1 generation			F2 generation	
		Cohort 1a	Cohort 1b	Cohort 3	PND 23	PND 45
Testes	XX	XX	XX	XX	XX	-
Epididymis	XX	XX	XX	-	-	-
Epididymis cauda	XX	XX	XX	-	-	-
Seminal vesicle (+/- fluid)	XX	XX	XX	-	-	-
Coagulating gland + fluid	XX	XX	XX	-	-	-
Prostate (dorsolateral and ventral separately)	XX	XX	XX	-	-	-
Vas deferens	X	X	X	-	-	-
Thymus	XX	XX	XX	XX	XX	-
Liver	XX	XX	XX	XX	XX	-

Organs examined	P generation	F1 generation			F2 generation	
		Cohort 1a	Cohort 1b	Cohort 3	PND 23	PND 45
Spleen	XX	XX	XX	XX	XX	-
Adrenal glands	XX	XX	XX	XX	XX	-
Kidneys	XX	XX	XX	XX	XX	-
Ovaries (L and R separately)	XX	XX	XX	-	-	XX
Uterus (wet/blotted)	XX	XX	XX	XX	XX	XX
Gravid uterus	-	XX	-	-	-	-
Brain	XX	XX	XX	XX	XX	-
Pituitary gland	XX	XX	XX	XX	XX	-
Thyroid (fixed >24 hr before weighing)	XX	XX	XX	XX	XX	XX
Lung	XX	XX	XX	-	-	-
Oviduct (Fallopian tube)	X	X	X	-	-	-
Heart	XX	XX	XX	-	-	-
Vagina	X	X	X	-	-	-
Cervix	X	X	X	-	-	-
Levator anibulbocavernosus muscle (LABC)	XX	XX	XX	-	-	-
Lymph nodes (mesenteric, inguinal)	X	X	X	-	-	-
Gross lesions	X	X	X	X	X	X
Bone marrow (sternum)	X	X	X	-	-	-
Spinal cord (cervical, thoracic, lumbar)	X	X	X	-	-	-
Sciatic nerve	X	X	X	-	-	-
Gastrocnemius muscle	X	X	X	-	-	-
Physical identifier	X	X	X	-	-	X
Mammary glands	X	X	X	-	-	-

b. Offspring: Pups that died or appeared moribund were subjected to gross necropsy. Lung flotation was performed on pups found dead on PND 0 to determine if the pup was stillborn. Pups culled on PND 4 had a gross necropsy and detailed visceral examination for developmental abnormalities.

c. F_1 Cohort 3 and F_2 offspring: Offspring were euthanized by carbon dioxide asphyxiation, and subjected to gross external and internal examination. F_1 Cohort 3 males and females were sacrificed on PND 70. F_2 offspring were sacrificed on PNDs 21, 23, and 45 (females only). The animals were weighed and examined macroscopically for abnormalities or pathological changes with special attention paid to the organs of the reproductive system.

As shown in the above table, the indicated (X) organs or tissues from F_1 Cohort 3 and F_2 offspring (PND 23) were preserved in appropriate fixative, and selected (XX) organs, in addition, were weighed. The ovaries, uterus, and thyroid gland were collected and weighed in F_2 female offspring on PND 45. Gross lesions were collected from all animals. Histopathology was conducted on the testis, liver, spleen, kidneys, uterus, and thyroid gland from the first 10-12 animals/sex in the control and high-dose groups of F_1 Cohort 3.

d. F_1 Cohort 1a fetal evaluations: The fetuses were sacrificed by injection of Fatal-Plus®. All fetuses were weighed, sexed, and examined for external malformations and variations on GD 20. Approximately half of the fetuses from each litter underwent visceral examination. The fetal thyroids were removed and fixed. The remaining half were examined for skeletal malformation and variations. The heads were sectioned according to the method of Wilson.

e. F_1 Cohort 2a (PND 70), 2b (PND 21), and F_2 (PND 21) pathology: Animals were weighed and perfused for fixation of nervous system tissues for neuropathology. The animals were anesthetized with 50 mg/kg pentobarbital and perfused via the left ventricle with sodium nitrite in phosphate buffer followed by 4% (w/v) EM-grade formaldehyde in phosphate buffer.

Brain were removed, measured, and weighed prior to fixation. The anterior-to-posterior lengths of the cerebrum and the cerebellum were measured from the anterior pole/edge to the posterior pole. In addition, all animals were examined macroscopically for structural abnormalities or pathological changes and special attention was paid to organs of the reproductive system.

Brains were processed for possible histopathology ((F_1 Cohorts 2a (PND70) and 2b (PND21))) and morphometric evaluations (all doses of F_1 Cohort 2a and the control and high-dose groups of F_1 Cohort 2b). Brain morphometrics were conducted on the frontal cortex, parietal cortex, caudate putamen, hippocampal gyrus, and cerebellum. Measurements recorded included thickness of the frontal and parietal cortex and the hippocampal gyrus, diagonal width of the caudate putamen, and height of the cerebellum.

The following sections of organs and tissues from F_1 Cohort 2a animals were preserved in 10% buffered formalin and processed for microscopic examination.

Brain levels 1-8	Gasserian ganglion
Brain levels 4, 5, 7	Eyes
Spinal cord- cervical	Optic nerve
Spinal cord- thoracic	Gastrocnemius muscle
Spinal cord- lumber	Peripheral nerves- sciatic
Cauda equine	Peripheral nerves- tibial
Spinal nerve root fiber & ganglia-cervical	Peripheral nerves- sural
Spinal nerve root fiber & ganglia-lumbar	

Taken from Appendix D, p. 1358, MRID 49547201.

D. DATA ANALYSIS:

1. Statistical analyses: Quantitative data were evaluated by Bartlett's test for homogeneity of variances. Homogeneous data were analyzed by analysis of variance (ANOVA) and Dunnett's *t*-test, and nonhomogeneous data were analyzed by Kruskal-Wallis ANOVA followed by Mann-Whitney U test, if differences were significant. Parametric data were analyzed by ANOVA followed by Dunnett's test if significant differences were found. Nonparametric data were analyzed by Kruskal-Wallis test followed by Dunn's test when significant differences were found. Nonparametric dichotomous data were analyzed by Chi-square test followed by Fisher's Exact test with Bonferroni adjustment when significant differences were found. Kruskal-Wallis nonparametric ANOVA was used to analyze proportional data, and Dunn's test was used when significant differenced were observed. Motor and locomotor activity and auditory startle response was analyzed using one-way or repeated-measures ANOVA followed by Dunnett's test. Continuous data from the FOB was analyzed using one-way ANOVA followed by Dunnett's test. Categorical data were analyzed using General Linear Modeling and Categorical Modeling Procedures with post-hoc Dunnett's test and Analysis of Contrasts, respectively. Ovarian follicle and corpora lutea counts and brain histo-morphometry data were analyzed by *t*-test. Semi-quantitative scores for thyroid follicular cell height and colloid area, determined for P males and females; F_1 males and females in Cohorts 1A, 1B and 3; F_2 PND 23 males and females; and PND 45 females at each dose level, were analyzed using a nonparametric Spearman correlation coefficient and p-value testing. The analysis was performed to determine whether there was a significant dose-related association of decreased colloid and increased follicle cell height. In addition, an analysis was performed to determine whether follicular cell height was correlated with T4 or TSH levels. All tests used a significance level of $p \leq 0.05$ or $p \leq 0.01$ except for Bartlett's test which used a significant level of $p \leq 0.001$.

2. **Indices:** The investigator calculated the following indices from the breeding and parturition records and the necropsy findings of the parental females in the study:

- $\text{Preimplantation Loss (\%)} = \left[\frac{\text{No. of corpora lutea - No. of implantations}}{\text{No. of corpora lutea}}\right] \times 100$
- $\text{Postimplantation Loss (\%)} = \left[\frac{\text{No. of implantations - No. of liveborn pups}}{\text{No. of implantations}}\right] \times 100$
- $\text{Mating Index (\%)} = \left[\frac{\text{No. of females that mated}}{\text{No. of females paired}}\right] \times 100$
- $\text{Fertility Index (\%)} = \left[\frac{\text{No. of pregnant females}}{\text{No. of females mated}}\right] \times 100$
- $\text{Gestation Index (\%)} = \left[\frac{\text{No. of females with liveborn pups}}{\text{No. of pregnant females}}\right] \times 100$

Gestation length was calculated as the number of whole days from insemination observed in the vaginal smear (GD0) to delivery of pups (LD0 parturition).
Offspring survival and viability indices: The investigator calculated the following indices from the lactation records of the litters:

- $\text{Birth Index (\%)} = \left[\frac{\text{No. of pups/litter}}{\text{Total No. of implantation sites/litter}}\right] \times 100$
- $\text{Live Birth Index (\%)} = \left[\frac{\text{No. of liveborn pups/litter}}{\text{Total No. of pups/litter}}\right] \times 100$
- $\text{Viability Index (\%)} = \left[\frac{\text{No. of pups/litter viable on PND 4 (pre-cull)}}{\text{No. of liveborn pups/litter}}\right] \times 100$
- $\text{Lactation Index (\%)} = \left[\frac{\text{No. of pups/litter viable on PND 21}}{\text{No. of pups/litter viable on PND 4 (post cull)}}\right] \times 100$

3. **Historical control data:** Historical control data for sperm analysis and vaginal opening in Wistar rats was provided from five studies. Sperm parameters included the mean motile and progressive sperm, mean testis and epididymal sperm counts (separately), and mean normal, abnormal, and detached head sperm from P and F_1 generations for each study and the range of means over all studies. The mean day of vaginal opening was presented for each study along with the range of means for all studies. The dates of the studies, source, age, and number of animals used, routes of administration, study types, and vehicle(s) used were not reported. Historical control data was also provided for brain morphometric data for Day 21 and PND 70 males and females from 25 studies. The dates of the studies, source of the animals, number of animals used, routes of administration, study types, and vehicle(s) used were not reported. Historical clinical pathology data (hematology, clinical chemistry, and urinalysis) from Wistar Hannover rats were included. The mean, standard deviation, number of animals, and range of parameters from 8-15 studies in males and females ranging in age from 7 to 60 weeks were provided. The dates of the studies, source of the animals, routes of administration, study types, and vehicle(s) used were not reported. Historical control data for developmental neurotoxicity auditory startle habituation and cognitive function and for neurotoxicity FOB

and motor activity testing were not in the report but were provided separately by the registrant.

4. **Peer Review of Selected Experimental Data:** Peer review was conducted by a consultant expert on developmental toxicity for fetal skeletal and head evaluation. Consultant pathologists conducted peer review of the thyroid semi-quantitative microscopic scoring observations and the brain morphometrics data. The conclusions presented in the study report were agreed upon by the study laboratory and the peer reviewers for each category of observation.

II. RESULTS:

A. PARENTAL ANIMALS: P generation

1. **Mortality and clinical observations:** There were no deaths or general clinical signs of toxicity related to treatment. One P parental female in the high-dose group was sacrificed *in extremis* on study day 73 due to unspecified illness. The animal was dehydrated and had labored breathing, rough coat, and nasal staining. Hepatocellular necrosis was observed and was possibly related to generalized infection/abscess found during necropsy.

2. **Body weight and food consumption:** Selected body weight and food consumption data for P parental males and females are given in Tables 5 and 6, respectively. There were no treatment-related effects on body weight, body weight gains, or food consumption in either sex. Females of the high-dose group had higher mean body weights compared with controls throughout the majority of the study period beginning on study day 0. Mean body weights for females in the high-dose group reached statistical significance on GD 20. Body weight gain during gestation was also statistically significantly higher than control during gestation.

Table 5. Selected mean body weight and weight gain values for P parental animals[a]

Study day	Dietary dose (ppm)			
	0	250	1000	2000
P Males (n = 30)				
Dose in mg/kg/day				
	0	13.9	53.2	106.7
Premating				
Day 0	326.8±3.96	323.1±3.72	325.9±3.64	324.0±3.97
Day 7	347.2±4.39	344.9±3.83	350.9±4.18	349.3±4.30
Day14	366.6±4.65	364.3±4.04	367.7±4.68	367.5±4.56
Day 28	398.3±5.88	394.2±4.39	396.7±5.17	398.5±5.40
Postmating				
Day 42	420.7±6.01	417.2±4.76	414.2±5.01	416.0±5.55
Day 56	441.2±6.55	435.7±5.28	430.5±5.18	432.7±6.06
Day 70	457.0±6.67	452.1±5.43	446.9±5.53	447.5±6.35
Day 84	463.0±6.60	458.8±5.67	457.2±5.86	457.7±6.37
P Females (n = 25-30)				
Dose in mg/kg/day				
	0	16.2	67.6	136.8
Premating				
Day 0	201.7±1.93	202.6±1.46	203.9±1.52	205.5±1.52
Day 7	207.6±1.77	208.1±1.67	206.0±1.38	210.1±1.74
Day 14	217.6±1.84	218.3±2.16	213.6±1.43	220.0±1.87
Day 28	225.1±2.07	227.5±2.04	223.6±1.87	230.0±1.92

Gestation				
GD 0	229.8±2.16	230.8±2.31	228.7±2.30	231.6±2.27
GD 6	248.5±2.21	251.4±2.42	248.3±2.25	253.2±2.23
GD 13	269.2±2.60	271.5±2.56	269.5±2.44	277.0±2.23
GD 20	326.3±4.45	334.3±3.02	334.0±3.44	342.9±4.82
Weight gain GD 0-20	96.5±3.31	103.5±2.19	105.3±2.33	111.3±3.97* (+15.3%)
Lactation				
LD 0	261.1±2.71	262.2±3.90	257.7±2.72	266.0±3.05
LD 7	281.1±3.24	282.5±2.61	283.1±2.55	287.6±2.90
LD 21	282.9±3.11	277.1±3.16	272.5±3.61	278.2±5.36

a Data extracted from Table 2, pp. 151-154; Table 9, p. 166 and Table 14, p. 171 of MRID 49547201. Values in parentheses indicate percentage change relative to controls. * statistically significant, $p<0.05$

Table 6. Mean food consumption for P generation parental animals (g±SD/animal/day)[a]

Study day	Dietary dose (ppm)			
	0	**250**	**1000**	**2000**
P Males (n = 30)				
Dose in mg/kg/day				
	0	**13.9**	**53.2**	**106.7**
Premating				
Days 0-7	70.8±1.04	74.1±1.72	70.0±0.96	71.2±1.04
Days 7-14	65.1±0.80	67.4±1.36	65.2±1.00	66.7±1.02
Days 14-21	63.9±0.98	67.0±1.68	63.9±1.31	63.9±1.01
Days 21-28	63.6±1.06	68.1±1.95	64.6±1.17	64.5±1.10
Postmating				
Days 42-49	59.4±1.01	63.7±1.95	60.3±1.37	60.4±0.96
Days 56-63	51.8±0.77	54.66±1.28	55.1±0.95	55.0±0.88
Days 77-84	46.8±0.77	49.7±1.31	47.2±0.81	47.6±0.90
P Females (n = 25-30)				
Dose in mg/kg/day				
	0	**16.2**	**67.6**	**136.8**
Premating				
Days 0-7	73.8±1.30	71.7±0.96	74.7±1.01	75.9±0.93
Days 7-14	71.9±1.67	72.2±1.20	74.1±1.10	74.1±1.07
Days 14-21	77.9±1.66	73.7±0.91	78.1±1.21	75.9±1.11
Days 21-28	71.2±1.75	69.8±0.85	73.9±1.66	72.2±1.29
Gestation				
GD 0-6	86.1±2.32	80.3±1.76	80.6±2.08	82.8±1.67
GD 6-13	84.8±1.53	80.5±1.14	83.9±1.36	82.8±1.16
13-20	87.4±2.12	83.9±1.72	84.5±1.64	83.3±1.28
Lactation				
LD 0-4	95.3±4.62	98.4±4.92	106.0±3.66	103.2±4.02
LD 4-7	143.0±3.39	142.1±2.74	146.2±4.01	145.3±4.62
LD 7-14	168.7±4.10	167.9±3.71	166.9±4.48	158.3±4.39
LD 14-21	225.7±8.46	216.7±5.71	227.0±6.93	226.1±8.93

a Data extracted from Table 4, p. 157, Table 11, p. 168, Table 16, p. 173 of MRID 49547201

3. **Test substance intake:** Mean test substance intake for the P parental and F_1 Cohort animals is shown in Table 4, above. These data are based on food consumption, body weight, and the nominal dietary concentrations of the test substance. There was no adjustment for purity, analytical results, or for increased consumption during lactation. The values for the P generation are considered to be representative of the test substance intake for the entire study, but actual intake for females during lactation was up to two-fold higher than in other life stages.

4. Clinical pathology:

a. Hematology: Selected hematology values are shown in Table 7. In P parental males of the high-dose groups, absolute monocyte count, percentage of monocytes, and mean cell hemoglobin concentration were decreased by 33% (n.s.), 29% ($p<0.05$), and 3% ($p<0.05$) respectively. The decreases were not considered adverse because the values were within the laboratory's historical control range, no changes were observed in other erythrocyte indices, and/or the magnitude of the change was small. These parameters were also not affected in hematology evaluations of F_1 Cohort 1a animals. No treatment-related effects were observed in females.

Table 7. Selected Hematology Parameters in P Adult Males and Females[a]

Parameter	Dietary dose (ppm)			
	0	**250**	**1000**	**2000**
Males (n = 10)				
Dose in mg/kg/day				
	0	**13.9**	**53.2**	**106.7**
Hematocrit (%)	47.7±1.8	48.3±1.3	48.0±.3	48.6±2.0
Hemoglobin (g/dL)	16.4±0.6	16.3±0.3	16.4±0.5	16.2±0.5
RBC (10^6/mm^3)	9.53±0.38	9.58±0.52	9.35±0.32	9.67±0.47
MCHC (g/dL)	34.4±0.4	33.9±0.8	34.1±0.6	33.4±0.8*
WBC (10^6/mm^3)	7.1±1.4	7.8±1.3	6.4±1.7	6.8±1.0
Monocytes (%)	3.4±0.9	3.3±0.8	3.2±1.0	2.4±0.5* (-29%)
Monocytes abs (10^6/mm^3)	0.24±0.07	0.26±0.09	0.21±0.10	0.16±0.05 (-33%)
Females (n = 10 - 11)				
Dose in mg/kg/day				
	0	**16.2**	**67.6**	**136.8**
Hematocrit (%)	49.8±2.0	50.1±2.1	48.6±4.1	49.1±2.5
Hemoglobin (g/dL)	16.7±0.7	17.0±0.5	16.2±1.3	16.7±0.7
RBC (10^6/mm^3)	8.93±0.41	9.07±0.44	8.66±0.95	8.92±0.56
MCHC (g/dL)	33.9±0.8	33.9±0.8	33.3±1.1	33.9±0.9
WBC (10^6/mm^3)	6.7±2.2	6.4±2.0	7.1±2.4	7.0±2.0
Monocytes (%)	3.7±1.0	3.6±1.0	3.4±0.9	3.0±0.8
Monocytes abs (10^6/mm^3)	0.25±0.13	0.23±0.11	0.24±0.11	0.22±0.10

a Data extracted from Tables 1-3 of pathology report (Appendix L), pp. 1666-1673 of MRID 49542701. Values in parentheses indicate percent change relative to controls. * statistically significant, $p<0.05$

b. Clinical chemistry: Selected clinical chemistry parameters are shown in Table 8. Alkaline phosphatase activity was significantly decreased (41%) in females of the mid-dose group, creatinine and alanine aminotransferase activity were significantly increased by 20 and 26%, respectively, in males of mid-dose group, and glucose was significantly increased (16%) in males of the high-dose group. The alterations were not considered to be adverse or related to treatment due to the lack of a clear dose response and the values were within the historical control range of the laboratory. Significantly increased triglycerides in females were also observed but were not considered adverse. Controls had some animals with triglyceride levels greater than the provided historical controls. These effects were also not seen in F_1 Cohort 1b animals.

Table 8. Selected Clinical Chemistry Parameters in P Adult Males and Females[a]				
Parameter	Dietary dose (ppm)			
	0	250	1000	2000
Males (n = 10) at PND 85				
Dose in mg/kg/day				
	0	**13.9**	**53.2**	**106.7**
Glucose (mg/dL)	122±9	132±23	133±10	142±11* (+16%)
Creatinine (mg/dL)	0.5±0	0.5±0	0.6±1* (+20%)	0.5±0.1*
Triglyceride (mg/dL)	71±27	62±21	66±28	49±13
AST (U/L)	70±9	70±10	69±8	76±18
ALT (U/L)	27±5	30±5	34±6* (+26%)	29±4
ALP (U/L)	67±15	69±18	65±8	81±22 (+21%)
Females (n = 10 - 11) at LD 22				
Dose in mg/kg/day				
	0	**16.2**	**67.6**	**136.8**
Glucose (mg/dL)	120.3±30	119±18	107±17 (-11%)	116±17
Creatinine (mg/dL)	0.6±0	0.5±0.1	0.6±0.1	0.6±0.1
Triglyceride (mg/dL)	147±103	75±21	81±89	67±30* (-54%)
AST (U/L)	110±27	97±13	130±36	113±25
ALT (U/L)	40±11	38±7	47±13	42±10
ALP (U/L)	83±20	62±30	49±18* (-41%)	65±22

a Data extracted from Table 7 of pathology report (Appendix L), pp. 1686-1689, MRID 49547201. Values in parentheses represent percent change relative to controls.
* statistically significant, $p<0.05$.

c. Urinalysis: There were no treatment-related effects on urine parameters in either sex.

d. Hormone data: Hormone data are presented in Table 9. Testosterone levels in males of the mid- and high-dose group were decreased by 25 and 24% (n.s.), respectively, compared with controls. The decreased levels were considered to be due to normal biologic variation because a dose-response was not observed, and reproductive performance, sperm parameters and accessory sex organ weights were not altered (slight testicular atrophy in 3 males and a 4% decrease in testes weight were seen but were not considered adverse due to the minimal nature of the changes). No treatment-related effects were observed on thyroid hormone levels in P males besides a 19% decrease (not significant) in T4 at 2000 ppm. In females, T4 levels were significantly increased at 250 and 1000 ppm (27 and 34%, respectively), and non-significantly (18%) at 2000 ppm. TSH levels were significantly increased in the females at 1000 ppm (164%) and non-significantly at 250 ppm (64%) and 2000 ppm (92%). Although thyroid hormone findings in P females did not show a clear dose-response and showed variability among the study cohorts, the hormonal changes in P females at 1000 and 2000 ppm were considered treatment-related based on the findings of thyroid follicular cell hypertrophy (see Table 11), and the semi-quantitative microscopic analysis of thyroid, which showed decreased colloid area and increased follicular cell height (see Table 45) at 1000 and 2000 ppm.

TABLE 9. Thyroid hormone and testosterone data for P parental animals [ab]				
Observation	Dose (ppm)			
	0	250	1000	2000
Males				
Dose in mg/kg/day	**0**	**13.9**	**53.2**	**106.7**
Testosterone (ng/dL)	190±135	187±193	142±144 (-25.3)[c]	145±152 (-23.7)
T4 (µg/dL)	4.21±0.89	3.87±0.72	3.59±0.83	3.42±0.62 (-18.7)
TSH (ng/mL)	1.30±0.56	1.25±0.51	1.38±0.70	1.29±0.26
Females				
Dose in mg/kg/day	**0**	**16.2**	**67.6**	**136.8**
T4 (µg/dL)	3.68±0.57	4.69±1.25* (27.4)	4.94±0.64* (34.2)	4.33±1.13 (17.7)
TSH (ng/mL)	1.31±0.34	2.15±1.11 (64.1)	3.46±2.65* (164)	2.51±1.25 (91.6)

[a] Data obtained from Text Table 21 (p. 82) and Appendix L Table 13 (p. 1721) and Table 15, p. 2104, MRID 49547201.

[b] Values are given as Mean ± Standard Deviation, n=12.

[c] Numbers in parentheses equal percent different from control; calculated by reviewer.

* Statistically different ($p < 0.05$) from the control.

5. P Parental postmortem results:

a. Organ weights: Selected absolute organ weights are shown in Table 10. No treatment-related effects were observed in the absolute or relative organ weight of P parental animals. In males, a 4% decrease (not statistically significant) in absolute testes weight was observed; relative testes weight was unaffected. Due to the small magnitude of the effect it was not considered adverse. In females of the low-dose group, absolute and relative spleen weights were significantly increased by 19 and 21%, respectively. The increases were not considered related to treatment because of the lack of dose-response relationship and lack of histopathological correlates.

Table 10. Absolute organ weights for P parental animals[a]				
Organ weight (g)	Dietary dose (ppm)			
	0	250	1000	2000
Males				
Dose in mg/kg/day				
	0	**13.9**	**53.2**	**106.7**
Terminal body weight (g)	467.6±37.2	463.6±31.3	456.6±32.4	455.9±35.9
Kidneys				
Left	1.445±0.132	1.417±0.106	1.368±0.100	1.377±0.143
Right	1.471±0.172	1.464±0.132	1.423±0.136	1.451±0.177
Liver	14.631±1.648	14.639±2.134	14.486±1.649	14.485±2.343
Testes				
Left	1.966±0.182	1.772±0.163	1.906±0.216	1.891±0.139 (-4%)
Right	1.939±0.177	1.871±0.163	1.872±0.228	1.863±0.147 (-4%)
Seminal vesicles	1.388±0.258	1.420±0.233	1.381±0.220	1.409±0.278
Epididymides				
Left	0.783±0.074	0.768±0.062	0.785±0.065	0.788±0.066
Right	0.766±0.67	0.735±0.071	0.748±0.065	0.746±0.071
Spleen	0.689±0.92	0.698±0.115	0.710±0.104	0.703±0.106
Thymus	0.423±0.108	0.452±0.088	0.465±0.112	0.433±0.096
Thyroid				
Left	0.014±0.003	0.014±0.003	0.014±0.003	0.014±0.003
Right	0.015±0.003	0.014±0.004	0.013±0.002	0.014±0.003

Table 10. Absolute organ weights for P parental animals[a]				
	Dietary dose (ppm)			
Organ weight (g)	**0**	**250**	**1000**	**2000**
Females				
Dose in mg/kg/day				
	0	**16.2**	**67.6**	**136.8**
Terminal body weight (g)	269.0±27.0	266.9±25.9	261.6±28.6	266.3±27.5
Kidneys				
Left	0.980±0.105	0.977±0.098	0.966±0.66	0.982±0.103
Right	1.029±0.102	1.034±0.108	1.035±0.114	1.038±0.118
Liver	12.239±2.381	12.109±2.694	12.398±2.90	12.246±2.528
Ovaries				
Left	0.051±0.009	0.059±0.015	0.058±0.013	0.058±0.011
Right	0.054±0.012	0.055±0.014	0.056±0.012	0.059±0.013
Uterus	0.792±0.327	0.682±0.208	0.602±0.134	0.670±0.171
Spleen	0.550±0.089	0.657±0.303* (+19%)	0.539±0.092	0.586±0.113
Thymus	0.244±0.073	0.245±0.055	0.227±0.076	0.259±0.096
Thyroid				
Left	0.012±0.002	0.012±0.002	0.012±0.003	0.012±0.002
Right	0.012±0.003	0.012±0.003	0.012±0.002	0.013±0.003

a Data extracted from Tables 26 and 27 of pathology report (Appendix L), pp. 2253-2300, MRID 49547201. N = 30/dose group. Values in parentheses indicate percent change relative to controls, calculated by reviewer.
* Statistically significant, $p<0.05$

b. Pathology:

1. **Macroscopic examination:** There were no treatment-related gross findings in P parental males or females.

2. **Microscopic examination:** Selected histopathological findings are summarized in Table 11. In males, slight or minimal testicular atrophy was seen in 1/30 low dose and 3/30 high dose males. The finding was not considered adverse because of the low incidence and severity, and lack of effects on sperm or male fertility. Hepatocellular necrosis was observed in 2/10 females of the high-dose group, but was not considered to be treatment-related because of the low incidence, no corresponding clinical chemistry findings, and an abscess/infection was observed in one of the animals. Minimal to slight thyroid follicular cell hypertrophy was observed in 2/29 females of the mid-dose group and 3/23 females in the high-dose group. Although the incidence and severity was low, it was considered treatment-related based on the increased TSH/T4 levels observed at both dose levels, along with the decreased colloid area/increased follicular cell height observed in the semi-quantitative microscope scoring data (Table 45).

Table 11. Selected histopathology findings for P parental animals[a]				
	Dietary dose (ppm)			
Tissue examined	**0**	**250**	**1000**	**2000**
Males				
Dose in mg/kg/day				
	0	**13.9**	**53.2**	**106.7**
Liver (N)	(10)	(0)	(0)	(10)
No abnormality	10	-	-	10
Testes (N)	(30)	(30)	(30)	(30)
No abnormality	30	29	30	26
Atrophy (grade)	0	1 (1.0)[b]	0	3 (1.3)
Degeneration	0	0	0	1 (1.0)
Vacuolation	0	0	0	1
Thyroid (N)	(26)	(28)	(29)	(30)
No abnormality	26	28	29	30
Females				
Dose in mg/kg/day				
	0	**16.2**	**67.6**	**136.8**
Liver (N)	(10)	(0)	(0)	(10)
No abnormality	10	-	-	8
Necrosis	0	-	-	2 (2.0)
Vacuolization, cytoplasm	0	-	-	1 (1.0)
Extramedullary hematopoiesis	0	-	-	1 (1.0)
Hemorrhage	0	-	-	1 (3.0)
Thyroid (N)	(26)	(28)	(29)	(23)
No abnormality	26	27	28	20
Hypertrophy, follicular cell	0	0	2 (1.5)	3 (1.0)
Cystic follicle	0	0	0	1 (1.0)
Infiltrate	0	1 (2.0)	0	0

a Data extracted from Table 55 of the pathology report (Appendix L), pp. 1810-1816 of MRID 49547201.
b Values in parentheses represent the mean severity grade (1.0 – minimal, 2.0, slight, 3.0 moderate)

6. Reproductive function and performance:

a. Estrous cycle length: Estrous cyclicity data are shown in Table 12. No significant difference was observed in mean estrous cycle length or the number of cycles in P females at any dose level compared to the control.

TABLE 12. Estrous cycle data for P parental females[ab]				
Estrous cycle length (days)	**Dose (ppm)**			
	0	**250**	**1000**	**2000**
	Dose in mg/kg/day			
	0	**16.2**	**67.6**	**136.8**
Mean length	3.8±0.3	4.0±0.2	4.2±0.2	4.1±0.1
Mean number of cycles	1.9±0.1	2.0±0.1	2.0±0.1	2.1±0.1

[a] Data obtained from Tables 126-127, pp. 444-445, MRID 49547201.
[b] Values are given as Mean ± Standard Error, n=30.

b. Terminal stage of estrous: Terminal stage of estrous in P females is shown in Table 13. No treatment-related findings were reported.

Table 13. Terminal stage of estrous in P females[a]				
Parameter	Dietary dose (ppm)			
	0	250	1000	2500
	Dose in mg/kg/day			
	0	16.2	67.6	136.8
Terminal stage of estrous				
Estrus (cornified cells)	4	4	5	7
Diestrus/metestrus (leukocytes)	25	26	25	23
Proestrus	0	0	0	0

a Data extracted from Table 140, p. 496 of MRID 49547201. N = 30, except that one control female was not examined for terminal state of estrous.

c. <u>Ovarian follicular counts</u>: Mean ovarian follicular counts of P females are shown in Table 14. No treatment-related effects were observed at high dose; therefore, low and mid dose were not evaluated.

Table 14. Mean ovarian follicular counts summary in P females[a]				
Ovarian follicle observation	Dietary dose (ppm)			
	Control	250	1000	2000
	Dose in mg/kg/day (based on P premating values)			
	0	16.2	67.6	136.8
-primordial follicle	49.85±17.79	-	-	51.55±13.46
-antral follicle	20.0±5.79	-	-	20.6±5.08
-corpus luteum	15.65±6.05	-	-	16.05±6.01

a Data extracted from text Table 18 on p. 78 of MRID 49547201; mean ±SD. N = 25-30.
- Data not available

d. <u>Sperm parameters</u>: Data on sperm parameters are shown in Table 15. There were no treatment-related effects on sperm motility, sperm counts, or sperm morphology. Sperm morphology was not evaluated in the low- and mid-dose groups due to lack of effects in the high-dose group.

TABLE 15. Sperm data for P parental males[ab]				
Parameter	Dose (ppm)			
	0	250	1000	2000
	Dose in mg/kg/day			
	0	13.9	53.2	106.7
% Motile sperm	94.7±2.6	93.2±4.1	94.0±2.9	94.7±2.6
% Progressive motile sperm	64.3±5.8	61.0±7.2	62.7±6.4	63.3±5.7
Testis sperm count (sperm/g)	30.0±6.9	30.5±9.0	33.3±8.8	31.6±6.1
Epididymis sperm count (sperm/g)	157.7±46.2	130.8±64.1	155.2±79.9	168.2±54.9
Mean total no. normal sperm	195.5±4.1	-	-	195.4±3.0
Mean total no. abnormal sperm	4.0±4.0	-	-	4.0±3.0
Mean total no. detached head sperm	0.5±0.7	-	-	0.5±0.8

[a] Data obtained from Text Table 20, p. 81, MRID 49547201.
[b] Values are given as Mean ± Standard Deviation, n=30.

e. <u>Reproductive performance</u>: The reproductive performances of the P parental animals are summarized in Table 16. There were no treatment-related effects on reproductive parameters.

TABLE 16. Reproductive performance of P parental animals[ab]				
Observation	**Dose (ppm)**			
	0	**250**	**1000**	**2000**
Dose in mg/kg/day (M/F)	**0/0**	**13.9/16.2**	**53.2/67.6**	**106.7/136.8**
Number of males/females paired	30/30	30/30	30/30	30/30
Number of pregnancies	30	29	27	27
Mating index (%)	100	100	96.7	96.7
Fertility index (%)	100	96.7	93.1	93.1
Gestation index (%)	100	100	100	100
Gestation length (days) Range of days	22.1±0.12 21-24	21.9±0.11 21-23	22.1±0.08 21-23	22.1±0.11 21-23
Precoital interval (days) Range of days	3.1±0.60 1-12	2.3±0.24 1-4	3.2±0.62 1-14	2.1±0.21 1-4
Total No. implantations Mean implantations/Dam	328 10.9±0.56	345 11.9±0.49	316 11.7±0.50	320 11.9±0.60

[a] Data obtained from Text Tables 18 and 19 (p. 78 and 79) and Table 7 (p. 164), MRID 49547201.

[b] Values are given as Mean ± Standard Error or Deviation

B. OFFSPRING: F_1

1. **Viability and clinical signs:** Litter parameters for the F_1 offspring are summarized in Table 17. No clinical observations attributed to treatment were observed. There were no treatment-related effects on the number of pups born, number of stillborn pups, sex ratio, anogenital distance, or viability indices. The number of pups and litters with pups found dead was increased in the high-dose group (control to high dose, 1, 1, 3 and 15 pups, respectively). The percentages of pups found dead were 0%, 0%, 3%, and 5% at 0, 250, 1000, and 2000 ppm, respectively and the number of litters with pups found dead for the same respective groups were 1, 1, 2, and 6. However, the increase at the high dose was due largely to pup loss from a single litter of 10 during lactation with the remaining 5 litters losing one pup each, and was therefore not considered a treatment-related effect.

TABLE 17. Litter parameters for F_1 offspring [a]

Observation	Dose (ppm)			
	0	250	1000	2000
Dose in mg/kg/day (M/F)(P premating intake)	**0/0**	**13.9/16.2**	**53.2/67.6**	**106.7/136.8**
Number of litters	30	29	27	27
Number of total litter resorptions	0	0	0	0
Number of litters born	30	29	27	27
Number with liveborn pups	30	29	27	27
Number with *any* stillborn pups	0	1	0	1
Number with pups found dead (%)	1 (3)	1 (3)	2 (7)	6 (22)[b]
Number of pups born: Total	297	328	303	298
Stillborn	0	4	0	1
Found dead (%)	1 (0)	1 (0)	3 (1)	15 (5)[b]
Mean litter size: PND 0	9.9±0.62	11.3±0.52	11.2±0.52	11.0±0.59
Mean number of live pups: PND 0	10	11	11	11
PND 4	10	11	11	11
PND 4 (post cull)	9	9	9	9
PND 21	9	9	9	9
Anogenital distance- cohort males (mm)	4.12±0.05	4.01±0.04	4.03±0.04	3.99±0.03
Anogenital distance- cohort females (mm)	2.27±0.04	2.23±0.03	2.24±0.05	2.28±0.03
Sex ratio (%male): PND 0	53.7±3.67	51.4±2.89	53.9±3.61	52.4±2.35
Birth index (%)	91.2±3.33	94.7±1.51	95.2±1.73	92.6±2.43
Live birth index (%)	100±0.00	98.5±1.53	100±0.00	99.7±0.34
Viability index (%)	94.7±3.57	99.7±0.27	98.2±0.74	98.2±0.75
Lactation index (%)	99.7±0.34	100±0.00	99.6±0.37	96.3±3.72

[a] Data obtained from Text Table 22 (p. 83) and Tables 19 and 23, (pp.176-177 and 188), MRID 49547201.

[b] The increase in pups found dead at 2000 ppm was largely due to complete litter loss in one dam (10 pups).

2. <u>Body weight</u>: Selected offspring body weight data are given in Table 18. Body weights of treated animals were not statistically different from body weights of control animals, although from ~ PND 14, body weights of males of the high-dose group were decreased by 6-7%, compared with controls. Although the decreases were not statistically significant, they are considered adverse effects based on the young age of the pups. Body weight gain of males in the high-dose group was significantly decreased from PND 4 through 18, by ~10%. Body weight gain of males in the mid-dose group was also significantly decreased by ~8% from PND 4 through 18, but no effects on body weight were seen. It is noted that the pups were likely exposed to higher test material intake during lactation due to greater food consumption by the lactating dams (the doses were not adjusted down during lactation), along with initiation of solid food feeding at about LD 14. The toxicokinetic evaluation in the range-finding study demonstrated that MBC was transferred to breast milk. However, female pups did not show decreases in body weight or weight gain.

TABLE 18. Mean (±SE) F_1 pup body weights (g) and body weight gain during lactation(g)[a]								
Observation Day	Dose (ppm)							
	0	250	1000	2000	0	250	1000	2000
	Dose in mg/kg/day							
	0	13.9	53.2	106.7	0	16.2	67.6	136.8
	F_1 pups – male				F_1 pups – female			
PND 0	6.2±0.08	6.1±0.11	6.0±0.10	6.0±0.09	5.8±0.10	5.7±0.12	5.7±0.09	5.7±0.08
PND 4[b]	10.3±0.24	9.9±0.29	10.0±0.23	10.0±0.23	9.8±0.26	9.5±0.30	9.6±0.20	9.6±0.21
PND 4[c]	10.3±0.24	9.9±0.29	10.0±0.24	10.0±0.23	9.7±0.26	9.4±0.31	9.6±0.19	9.5±0.21
PND 7	15.8±0.34	15.4±0.38	15.4±0.35	15.2±0.40	15.0±0.33	14.7±0.41	14.6±0.26	14.5±0.38
PND 14	30.1±0.60	29.8±0.76	28.5±0.59	28.0±0.59 (-6.3)	28.3±0.50	28.7±0.80	27.3±0.48	27.2±0.52
PND 18	38.4±0.81	37.6±0.99	36.0±0.79	35.6±0.76 (-9.3)	36.0±0.65	36.2±0.97	34.4±0.61	34.4±0.72
PND 21	48.6±0.99	47.5±1.19	46.2±1.09	45.6±0.95 (-6.2)	45.6±0.97	45.5±1.17	44.1±0.89	43.8±0.86
Body weight gain								
PND 4-18	28.2±0.66	27.7±0.79	26.0±0.61*(-7.8)[d]	25.5±0.69*(-9.6)	26.3±0.49	26.8±0.77	24.8±0.50	24.8±0.63
PND 0-21[e]	42.4	41.4	40.2	39.6	39.8	39.8	38.4	38.1

[a] Data from Tables 21 and 22, pp. 179-181, MRID 49547201.
[b] Before culling.
[c] After (culling).
[d] Numbers in parentheses equal percent different from control; calculated by reviewer.
[e] Calculated by reviewer.
* Statistically different ($p < 0.05$) from the control.

3. Developmental landmarks:

a. **Nipple/areola retention:** There were no treatment-related effects on nipple/areola retention among the groups of F_1 males.

b. **Anogenital distance:** No treatment-related effects on anogenital distance were observed in males or females (Table 19).

Table 19. Anogenital distance in F_1 offspring (pups selected from all cohorts)[a]				
Parameter	Dietary dose (ppm)			
	Control	250	1000	2000
Males (N = 26-30)				
Dose in mg/kg/day	0	13.9	53.2	106.7
Anogenital distance (mm ±S.E.)	4.12±0.05	4.01±0.04	4.03±0.04	3.99±0.03
Females (N = 27-29)				
Dose in mg/kg/day	0	16.2	53.2	106.7
Anogenital distance (mm ±S.E.)	2.27±0.04	2.23±0.03	2.24±0.05	2.28±0.03

[a] Data extracted from Table 23, p. 188, MRID 49547201.

4. **Hormones:** Hormone data for F_1 pups on PND 4 (blood samples pooled by litter) and Cohort

3 pups on PND 23 (samples collected from individual pups) are shown in Table 20. On PND 4, TSH was unchanged across dose groups. In the high-dose group, T4 levels were significantly decreased in F_1 pups on PND 4 at 2000 ppm (34.5%, $p<0.05$) and non-significantly decreased at 1000 ppm (24%). The study author did not consider the decrease to be related to treatment because a consistent pattern in thyroid hormone levels was not observed across the PND life stages. However, the decreases are also associated with thyroid hormone changes and statistically significant changes in the semi-quantitative analysis of colloid area and follicular and thyroid effects in the P females at the same doses, and are therefore considered treatment-related. Thyroid histopathology of the PND 4 pups was not performed in this study.

No statistically significant differences were observed among F_1 Cohort 3 females on PND 23, although T4 and TSH values were increased (22% and 35%) at 2000 ppm. T4 and TSH levels were significantly increased (53% and 41%) in F_1 males of the high-dose group on PND 23. T4 was also significantly increased (42%) and TSH non-significantly increased (21%) in males of mid-dose group on PND 23. The study author did not consider the changes to be treatment-related or biologically significant because a statistically significant correlation between follicle cell height scores and TSH/T4 hormone alterations was not observed, and there was no consistent pattern of effect on F_2 pups (see Table 43). However, statistically significant changes in the semi-quantitative analysis of colloid area and follicular cell height were observed in males at 1000 ppm and both sexes at 2000 ppm (see Table 46). Therefore, these alterations were considered treatment-related.

TABLE 20. Thyroxin (T4) and thyroid stimulating hormone (TSH) data for F_1 PND 4 pups and F_1 Cohort 3 PND 23 pups [ab]

Observation	Dose (ppm)			
	0	250	1000	2000
	Dose in mg/kg/day (M/F)			
	0/0	13.9/16.2	53.2/67.6	106.7/136.8
PND 4 (F_1 pups pooled)				
T4 (µg/dL)	0.84±0.28	0.71±0.24	0.64±0.22 (-24)[c]	0.55±0.14* (-34.5)
TSH (ng/mL)	0.49±0.14	0.61±0.14	0.48±0.13	0.52±0.16
PND 23 (F_1 Cohort 3)				
Males				
T4 (µg/dL)	2.64±0.47	2.89±0.57	3.74±0.87* (+41.7)	4.03±0.71* (+52.7)
TSH (ng/mL)	0.58±0.12	0.64±0.19	0.70±0.18 (+21)	0.82±0.23* (+41.4)
Females				
T4 (µg/dL)	2.79±0.65	2.25±0.84	2.81±0.52	3.41±0.82 (+22)
TSH (ng/mL)	0.43±0.14	0.49±0.23	0.55±0.16 (+28)	0.58±0.20 (+35)

[a] Data obtained from Text Table 23 (p. 85) and Appendix L Text Table G (p. 1643), MRID 49547201.

[b] Values are given as Mean ± Standard Deviation, n=12 for all groups.

[c] Numbers in parentheses equal percent different from control; calculated by reviewer.

* Statistically different ($p<0.05$) from the control.

5. <u>Sexual maturation</u>: No treatment-related effects were observed on preputial separation or vaginal opening in F_1 animals (Table 21). A slight increase in the time to vaginal opening was observed in females of the high-dose group (+1.9 days), but the value was reported to be within the historical control data range.

Table 21. Sexual maturity of F_1 offspring (Cohorts 1a, 1b, and 2a)[a]				
Parameter	**Dose (ppm)**			
	0	**250**	**1000**	**2000**
Preputial separation	**Males**			
Age (days)	51.0±4.0	50.6±3.8	49.3±2.7	52.0±9.2
Body weight (g)	232±29.8	229±28.1	223±25.9	226±24.4
Vaginal opening	**Females**			
Age (days)	32.1±2.5	32.8±6.3	32.4±2.9	34.0±3.3
Body weight (g)	97±14.1	97±21.4	96±15.1	103±16.9

[a] Data from Tables 44 and 48, pp. 212 and 216, MRID 49547201.

[b] Values are given as Mean ± Standard Deviation, n=49-51/group

6. Offspring postmortem results: F_1 PND 4 culls, PND 21 pups not assigned to Cohort, and Cohort 3

a. **Organ weights:** No treatment-related organ weight changes were observed in Cohort 3 males (Table 22). Statistically significantly increased relative liver weights (10%) were observed in F_1 Cohort 3 females of the high-dose group. The increase was observed in the absence of histopathological findings. In females of the mid-dose group, absolute and relative pituitary weights were decreased by 40% and 31% (n.s.), respectively, but were not considered related to treatment due to a lack of a dose-response relationship or histopathological changes.

Table 22. Absolute and relative organ weights for F_1 offspring Cohort 3[a]				
Organ weight (g)	**Dietary dose (ppm)**			
	0	**250**	**1000**	**2000**
Males (N = 12), PND 23				
Dose in mg/kg/day				
	0	**13.9**	**53.2**	**106.7**
Terminal body weight (g)	55.4±4.7	54.6±5.1	56.2±4.1	52.8±5.1 (-4.7%)
Liver abs	2.348±0.246	2.220±0.220	2.451±0.308	2.268±0.300
rel	4.232±0.206	4.067±0.168	4.356±0.295	4.289±0.246
Testes				
Left abs	0.171±0.024	0.165±0.018	0.171±0.013	0.158±0.019
rel	0.308±0.033	0.302±0.026	0.305±0.010	0.300±0.023
Right abs	0.167±0.174	0.161±0.017	0.170±0.018	1.839±0.150
rel	0.301±0.030	0.295±0.023	0.303±0.020	0.295±0.024
Brain abs	1.485±0.041	1.487±0.057	1.490±0.079	1.461±0.05
rel	2.695±0.222	2.740±0.205	2.661±0.113	2.790±0.260
Thymus abs	0.208±0.037	0.207±0.030	0.204±0.032	0.186±0.045
rel	0.374±0.050	0.381±0.061	0.361±0.037	0.348±0.059
Thyroid (left + right)				
abs	0.009±0.003	0.009±0.004	0.008±0.003	0.009±0.003
rel	0.0161±0.0043	0.0170±0.0072	0.0139±0.0054	0.0176±0.0056
Pituitary abs	0.004±0.001	0.004±0.001	0.004±0.001	0.004±0.002
rel	0.0065±0.0021	0.0069±0.0023	0.0067±0.0027	0.0073±0.0040

Table 22. Absolute and relative organ weights for F_1 offspring Cohort 3[a]				
	Dietary dose (ppm)			
Organ weight (g)	**0**	**250**	**1000**	**2000**
Females (N = 12), PND 23				
Dose in mg/kg/day				
	0	**16.2**	**67.6**	**136.8**
Terminal body weight (g)	54.1±3.5	52.5±7.6	52.2±8.6	52.3±7.3
Liver abs	2.242±0.142	2.200 ±0.299	2.257±0.343	2.406±0.538
rel	4.149±0.205	4.201±0.205	4.347±0.361	4.547±0.462*(+10%)
Brain abs	1.462±0.052	1.444±0.062	1.423±0.057	1.404±0.080
rel	2.708±0.137	2.798±0.381	2.775±0.345	2.731±0.398
Thymus abs	0.220±0.027	0.203±0.051	0.194±0.047	0.183±0.031
rel	0.407±0.058	0.382±0.062	0.369±0.036	0.352±0.049
Thyroid (left + right)				
abs	0.010±0.002	0.010±0.004	0.010±0.004	0.010±0.002
rel	0.0181±0.0045	0.0184±0.0074	0.0187±0.0075	0.0218±0.0066
Pituitary abs	0.005±0.002	0.004±0.001	0.003±0.001*(-40%)	0.004±0.001
rel	0.0094±0.0039	0.0072±0.0025	0.0065±0.0026	0.0084±0.0024

a Data extracted from Tables 32-33 and 49-50 of pathology report (Appendix L), pp. 1755-1758, 1796-1799 of MRID 49547201.

b. Pathology:

1. **Macroscopic examination:** There were no treatment-related gross findings in males or females pups on PND 4 or 21. A low incidence of renal pelvis dilatation was observed in both sexes but did not show a dose-response (see Table 26, below).

2. **Microscopic examination:** There were no treatment-related microscopic changes in the thyroid glands or reproductive organs of Cohort 3 animals. However, a semi-quantitative analysis of the thyroid histopathology (see Section G) showed significantly increased thyroid follicular height and decreased colloid area on PND 23 in both sexes at 1000 and 2000 ppm, described below (Table 46, below).

C. F_1 COHORT 1A REPRODUCTIVE AND SYSTEMIC TOXICITY:

1. **Mortality and clinical observations:** No treatment-related clinical signs were observed. One female each was found dead in the control (PND 124), low- (PND 125), and mid-dose (PND 42) groups. The deaths were not considered to be related to treatment due to lack of a dose-response.

2. **Body weight and food consumption:** Selected body weight data are given in Table 23. There was no effect of treatment on body weight, body weight gain, or food consumption in females. Gravid uterine weight, net maternal body weight and net weight gain showed no treatment-related changes. In the high-dose group, body weights of males on PNDs 24, 27, and 28 were decreased (9-10%) compared to controls, continuing the trend for lowered body weight that was observed prior to weaning. The decreased body weights observed on PNDs 27 and 28 were statistically significantly different from controls. These decreases were considered adverse due to the young age of the post-weaning pups, but the body weights recovered and were similar to controls at later time points. It is noted that during lactation pups may have had increased exposure to test material due to increased food consumptions of the dams during lactation, along with the onset of solid food intake by the pups. Food consumption in males was significantly increased during study days 42-49 and 63-70, but the increases did not alter body weight gain or mean body weights.

TABLE 23. Mean body weight, body weight gain, and food consumption for F_1 Cohort 1a animals[ab]

Interval	Dose (ppm)			
	0	250	1000	2000
F_1 Cohort 1a males				
Dose in mg/kg/day				
	0	**13.9**	**53.2**	**106.7**
Body weight (g)				
PND 24	64.2±1.1	61.0±1.7	63.2±1.6	58.7±1.4 (-8.6)[c]
PND 27	80.5±1.1	76.8±1.9	80.0±1.7	72.5±2.3** (-9.9)
PND 28	86.9±1.4	83.6±1.9	86.4±1.8	78.1±2.6** (-10.1)
Day 0[d]	144.3±3.91	149.1±3.44	147.4±3.51	137.2±3.42
Day 70	403.2±8.50	401.5±8.11	404.8±5.9	397.8±7.09
Day 112	438.5±26.39	475.6±23.76	437.1±7.51	422.5±9.10
Body weight gain (g)				
Day 0-112[e]	377.3	414.6	373.9	363.8
Food consumption (g/kg/day)				
Days 0-7	143±5.90	141.2±2.04	141.4±2.37	150.8±2.88
Days 42-49	64.8±0.70	64.9±0.80	64.0±1.08	68.4±0.94** (5.6)
Days 63-70	55.7±0.65	55.0±0.65	56.5±0.97	58.5±0.69* (5)
Days 105-112	53.1±1.30	51.7±0.73	53.7±2.34	53.1±1.03
F_1 Cohort 1a females				
Dose in mg/kg/day				
	0	**16.2**	**67.6**	**136.8**
Body weight (g)				
PND 24	59.3±1.3	55.0±1.7	57.4±1.3	58.1±1.2
PND 27	72.7±1.3	71.2±1.6	70.3±1.5	71.5±1.3
PND 28	77.9±1.4	75.8±1.6	75.1±1.5	77.7±1.3
Day 0[d]	114.4±2.8	115.4±1.54	112.3±2.51	115.6±2.31
Day 70	220.1±3.63	219.2±3.39	223.8±2.93	226.4±4.50
GD 0	220.6±4.05	218.9±3.50	223.3±2.91	224.7±4.45
GD 20	318.3±6.20	319.3±4.87	324.5±4.00	328.3±5.19
Body weight gain (g)				
Day 0-70[e]	105.7	103.8	111.5	110.8
GD 0-20	97.7±3.43	99.1±3.64	101.3±2.94	103.5±2.45
GD 0-20 (excluding uterine wt)	38.7±1.68	36.4±1.78	40.2±2.09	38.7±2.42
Gravid uterine weight and net body weight (g)				
GD 20 uterine weight	58.9±2.52	62.9±2.71	61.1±2.63	64.7±2.13
GD 20 net body weight	259.4±4.65	256.3±3.42	263.4±3.25	264.0±4.26
Food consumption (g/kg/day)				
Days 0-7	144.6±4.16	140.3±2.96	154.4±5.44	149.3±6.15
Days 42-49	77.0±1.08	79.8±1.14	79.7±1.21	78.8±1.08
Days 63-70	71.8±1.09	72.8±1.50	74.6±1.77	72.5±1.03
GD 0-6	82.6±3.92	73.9±1.85	81.5±4.86	85.5±4.22
GD 18-20	65.7±1.99	64.6±1.34	69.8±2.14	66.7±1.79

[a] Data obtained from Table 37 (pp. 202-203), Table 53 (pp. 222-225), Tables 59-61 (pp. 237-240), and Table 65 (p. 244), MRID 49547201.

[b] Values are given as Mean ± Standard Error, n=22/group except Day 112, n=5/group.

[c] Number in parenthesis equal percent change, relative to control value, calculated by the reviewer.

[d] Animals were PND 33-39 on Day 0.

[e] Calculated by reviewer

* Statistically different ($p < 0.05$) from the control.

** Statistically different ($p < 0.01$) from the control.

3. Hormones: Thyroid hormone data are presented in Table 24. There were no treatment-related effects on thyroid hormones in females at GD 20.

TABLE 24. Thyroid hormone data in F_1 Cohort 1a females on GD 20 [ab]

Observation	Dose (ppm)			
	0	250	1000	2000
	Dose in mg/kg/day			
	0	16.2	67.6	136.8
Females				
T4 (µg/dL)	1.52±0.67	2.07±0.64	1.94±0.54	1.91±0.83
TSH (ng/mL)	2.53±1.55	2.40±1.87	2.62±1.5	1.77±1.16 (-30)
T3 (ng/mL)	49.04±12.55	45.34±12.7	43.90±7.68	47.06±8.55

[a] Data obtained from Text Table 24, p. 88, MRID 49547201.

[b] Values are given as Mean ± Standard Deviation, n=12 for all groups.

4. Postmortem results:

a. Organ weights: Selected absolute and relative (to body) organ weight data for F_1 Cohort 1a animals at PND 85 (males) and GD 20 (females) are shown in Table 25. Significantly increased absolute right and left testis weights were observed in the mid- and high-dose males. Decreased seminal vesicle weights (without fluid) were also observed in the high-dose group. The findings were not considered treatment-related because of the lack of dose-response relationship and gross pathology or sperm evaluation findings, relative testis weights were not statistically different, seminal vesicle weights (with fluid) were not different among groups, similar weight changes and histopathological findings were not observed in the reproductive tissues of males of the F_1 Cohort 1b which were sacrificed ~30 days after F_1 Cohort 1a males.

Increases in absolute (n.s.) and relative (s.s.) thymus weights were observed in males of the high-dose group. In females of the mid- and high-dose groups, absolute and relative thymus weights were significantly increased. Liver weights (absolute and relative) showed very slight increases in both males and females (relative liver weight was significantly increased in males), but all changes were ≤7% of control mean weights. There were no gross pathology findings and no correlating weight changes or histopathological findings in F_1 Cohort 1b animals; therefore, the findings were considered to be incidental. No other changes in organ weights were observed, including the thyroid for both males and females.

TABLE 25. Selected organ weight data for F_1 Cohort 1a animals[ab]

Observations	Dose (ppm)			
	0	250	1000	2000
Males (PND 148)				
Dose in mg/kg/day				
	0	**13.9**	**53.2**	**106.7**
Terminal body wt (g)	454.3±43.8	451.0±44.9	450.6±30.4	449.0±38.0
Absolute liver wt (g)	15.792±2.075	15.578±2.088	16.092±1.946	16.557±1.775 (+4.8)
Organ/body ratio	3.476±0.288	3.449±0.238	3.567±0.317	3.688±0.241* (+6)
Absolute thyroid right/left (g)	0.016±0.003/ 0.016±0.003	0.017±0.002/ 0.016±0.005	0.016±0.002/ 0.015±0.004	0.016±0.003/ 0.015±0.003
Organ/body ratio right/left	0.004±0.001/ 0.004±0.001	0.004±0.001/ 0.003±0.001	0.003±0.00/ 0.003±0.001	0.004±0.001/ 0.003±0.001
Absolute seminal vesicle wt (g)	0.618±0.085	0.605±0.118	0.583±0.089	0.537±0.075* (-13.1)[c]
Organ/body ratio	0.137±0.022	0.135±0.029	0.129±0.017	0.120±0.014* (-12.4)
Absolute thymus wt (g)	0.505±0.117	0.510±0.131	0.532±0.100	0.553±0.087 (+9.5)
Organ/body ratio	0.111±0.023	0.113±0.031	0.118±0.020	0.124±0.020* (+11.7)
Absolute right testis wt (g)	1.946±0.141	1.866±0.161	1.793±0.165* (-7.9)	1.832±0.119* (-5.9)
Organ/body ratio	0.432±0.053	0.416±0.045	0.398±0.032	0.411±0.043
Absolute left testis wt (g)	1.950±0.140	1.871±0.152	1.817±0.164* (-6.8)	1.833±0.118* (-6)
Organ/body ratio	0.433±0.053	0.418±0.046	0.404±0.035	0.411±0.041 (-5.1)
Females (GD 20)				
Dose in mg/kg/day				
	0	**16.2**	**67.6**	**136.8**
Terminal body wt (g)	318.4±28.0	319.0±21.9	324.8±17.3	325.9±25.5
Absolute liver wt (g)	11.976±1.359	11.810±1.227	12.465±1.090	12.841±1.658 (+7.2)
Organ/body ratio	3.762±0.262	3.698±0.215	3.839±0.282	3.932±0.297 (+4.5)
Absolute thyroid right/left (g)	0.012±0.002/ 0.011±0.002	0.012±0.002/ 0.011±0.001	0.012±0.002/ 0.012±0.003	0.012±0.003/ 0.012±0.002
Organ/body ratio right/left	0.004±0.001/ 0.004±0.001	0.004±0.001/ 0.004±0.00	0.004±0.001/ 0.004±0.001	0.004±0.001/ 0.004±0.001
Absolute thymus wt (g)	0.300±0.038	0.338±0.044	0.354±0.052* (+18)	0.364±0.068* (+21.3)
Organ/body ratio	0.095±0.011	0.106±0.015	0.109±0.017* (+14.7)	0.113±0.026* (+18.9)

[a] Data obtained from Appendix L Tables 28-29 (pages 1741-1747) and Tables 45-46 (pages 1781-1787), MRID 49547201.

[b] Values are given as Mean ± Standard Deviation; n= 22 males, 20 females for all groups.

[c] Numbers in parentheses equal percent change, relative to control value, calculated by reviewer.

* Statistically different ($p < 0.05$) from the control.

b. Pathology:

1. **Macroscopic examination:** There were no treatment-related gross necropsy findings in 1a males or females. Renal pelvic dilatation was observed in all dose groups. In males the incidence was increased at 1000 and 2000 ppm, but in females it was highest in controls, and it did not show a dose-related increase in Cohorts 1b or 3. Incidences for Cohorts 1a, 1b and 3 are shown in Table 26.

Table 26. Gross observation of renal pelvis dilation in Cohorts 1a, 1b and 3[a]				
Group and severity of finding	**Dietary dose (ppm)**			
	0	**250**	**1000**	**2000**
Males				
Dose in mg/kg/day				
	0	**13.9**	**53.2**	**106.7**
Cohort 1a				
Mild	2	2	5	8
Moderate	1	1	3	3
Cohort 1b				
Mild	0	2	2	0
Moderate	0	1	2	1
Marked	0	0	1	0
Cohort 3				
Mild	1	0	2	1
Moderate	0	0	2	1
Females				
Dose in mg/kg/day				
	0	**16.2**	**67.6**	**136.8**
Cohort 1a				
Mild	3	1	1	2
Moderate	1	1	0	0
Cohort 1b				
Mild	0	2	1	2
Moderate	0	0	0	0
Cohort 3				
Mild	0	2	0	0
Moderate	0	0	0	1

a Data extracted from Tables 93-97 of pathology report (Appendix L), pp. 2169 to 2201 of MRID 49547201.

2. **Microscopic examination:** No treatment-related histopathological findings were observed in the reproductive organs of treated males. Pathology was not conducted in females. Low incidences of renal pelvic dilatation were observed in males and females of all groups.

5. Reproductive parameters:

a. Cohort 1a estrous cycle: Data are shown in Table 27. There were no differences in the interval between vaginal opening and first estrus, mean estrous cycle length, or estrous cycle pattern among the groups.

TABLE 27. Estrous cycle data for F_1 Cohort 1a parental females[ab]				
Estrous cycle (days)	**Dose (ppm)**			
	0	**250**	**1000**	**2000**
	Dose in mg/kg/day			
	0	**16.2**	**67.6**	**136.8**
After vaginal opening				
Mean length	5.2±0.2	5.5±0.4	4.9±0.1	4.7±0.1
Mean number of cycles	2.8±0.1	2.7±0.2	2.9±0.2	3.0±0.2
Premating				
Mean length	3.8±0.4	4.1±0.3	4.5±0.4	4.3±0.4
Mean number of cycles	1.6±0.2	1.6±0.2	1.4±0.1	1.6±0.2

[a] Data obtained from Table 57, p. 235, MRID 49547201.

[b] Values are given as Mean ± Standard Error, n=20-22

b. Cohort 1a sperm parameters: Data are shown in Table 28. There were no treatment-related effects on sperm motility, epididymal sperm counts, or sperm morphology. Testicular sperm counts in the high-dose group were significantly increased by 37%, compared to control, but the effect is not considered adverse.

TABLE 28. Sperm data for F_1 Cohort 1a parental males[ab]

Parameter	Dose (ppm)			
	0	250	1000	2000
Dose in mg/kg/day	**0**	**13.9**	**53.2**	**106.7**
% Motile sperm	93.8±3.7	95.4±1.4	94.7±1.9	94.8±2.0
% Progressive sperm	56.5±7.8	60.7±7.8	60.9±4.4	62.5±5.0
Testis sperm count (sperm/g)	32.4±13.5	32.3±10.8	41.2±11.2	44.3±10.1* (36.7)[c]
Epididymis sperm count (sperm/g)	157.3±59.1	169.7±65.8	199.5±63.6	232.3±70.5
Mean normal sperm	198.2±1.1	-	-	198.1±1.9
Mean abnormal sperm	1.3±1.2	-	-	1.6±2.0
Mean detached head sperm	0.5±0.8	-	-	0.3±0.5

[a] Data obtained from Text Table 26, p. 92, MRID 49547201.

[b] Values are given as Mean ± Standard Deviation, n=10.

[c] Numbers in parentheses equal percent change, relative to control value, calculated by reviewer.

* Statistically different ($p < 0.05$) from the control.

\- Not analyzed

c. Cohort 1a reproductive performance: The reproductive performances of the F_1 Cohort 1a parental animals are summarized in Table 29. There were no treatment-related effects on reproductive indices, number or corpora lutea, resorptions, or implantation, pre- or post-implantation loss, or sex ratio. Mean litter sizes and weights were similar among the groups.

TABLE 29. Reproductive performance of F_1 Cohort 1a parental animals[a]

Observation	Dose (ppm)			
	0	250	1000	2000
Dose in mg/kg/day (M/F)	**0/0**	**13.9/16.2**	**53.2/67.6**	**106.7/136.8**
Number of pregnancies	20	21	18	19
Mating index (%)	100	95.5	100	100
Fertility index (%)	100	100	100	95.5
Gestation index (%)	100	100	100	100
Maternal wastage				
No. Died	1	1	0	0
No. Aborted/delivery early	0	0	0	0
Total No. corpora lutea	296	286	254	276
Corpora lutea/Dam	14.8±0.61	13.6±0.54	14.1±0.84	14.5±0.45
Total No. implantations	229	240	200	230
Implantations/Dam	11.4±0.46	11.4±0.43	11.1±0.43	12.1±0.35
Total preimplantation loss	67	46	54	46
Mean loss/animal	3.3±0.62	2.2±0.43	3.0±1.04	2.4±0.45
Preimplantation loss (%)	21.2±3.30	15.3±2.65	18.1±3.96	15.9±2.53
Total postimplantation loss	19	6	9	9
Mean loss/animal	0.9±0.29	0.3±0.12	0.5±0.17	0.5±0.14
Postimplantation loss (%)	8.3±2.49	3.0±1.26	4.9±1.84	4.01.22
Total resorptions/dam	0.9±0.29	0.3±0.12	0.5±0.17	0.5±0.14
Total resorptions	19	6	9	9
Early resorptions/dam	0.8±0.2	0.3±0.12	0.5±0.17	0.4±0.14
Total early resorptions	15	6	9	8
Late resorptions/dam	0.2±0.16	0.0±0.00	0.0±0.00	0.1±0.05
Total late resorptions	4	0	0	1
Total No. live fetuses	210	234	191	221
Live fetuses/Dam	10.5±0.48	11.1±0.49	10.6±0.49	11.6±0.38
Total No. dead fetuses	0	0	0	0
Mean fetal weight combined	3.6±0.06	3.6±.0.06	3.5±0.07	3.5±0.05
Males	3.7±0.06	3.7±0.05	3.6±0.08	3.5±0.06
Females	3.5±0.07	3.5±0.06	3.4±0.08	3.4±0.05
Number of males/litter	5.3±0.33	5.8±0.51	5.8±0.50	6.3±0.45
Total number of males	106	122	104	120
Number of females/litter	5.2±0.44	5.3±0.46	4.8±0.44	5.3±0.33
Total number of females	104	112	87	101
Fetal sex ratio (median % male)	52	50	55	55

[a] Data obtained from Tables 66-67, pp. 245-247, MRID 49547201.

[b] Values are given as Mean ± Standard Error.

d. Cohort 1a developmental toxicity (GD20 fetal examination):

1. **External examination**: Fetal external observations are presented in Table 30a. No treatment-related external malformations or variations were observed. One malformation, absent eye bulge on the left side, was observed in one fetus at 1000 ppm which was considered an incidental finding since no dose-response was observed.

3. **Visceral examination:** Visceral observations are presented in Table 30. No treatment-related visceral malformations or variations were observed. Anomalous pattern of major vessels was observed in the same mid-dose animal with an absent eye bulge; the finding was at a slightly higher incidence in the treated groups compared to controls, but did not show a clear dose-response or a significant increase. All other findings were incidental and observed across all groups.

3. **Skeletal examination:** Selected skeletal observations are presented in Table 30c. No treatment-related skeletal malformations or variations were observed. A statistically significant increase in the percentage of affected fetuses/litter with unossified caudal arches with cartilage present (variation) was observed in the mid- and high-dose groups. This effect is not considered adverse, as delayed ossification of the caudal arches (with or without cartilage) was observed in almost all fetuses of essentially all litters at all dose levels including controls. This finding with cartilage present is not considered as a distinct finding independent of the delayed ossification of caudal arches without cartilage.

TABLE 30a. External examinations, F_2 offspring from Cohort 1a [a]

Observations	Dose (ppm)			
	0	250	1000	2000
	Dose in mg/kg/day (M/F)			
	0/0	13.9/16.2	53.2/67.6	106.7/136.8
No. Fetuses(litters) examined	210 (20)[b]	234 (21)	191 (18)	221 (19)
Malformations/variations				
No eye bulge on left side	0 (0)	0 (0)	1 (1)	0 (0)

[a] Data obtained from Table 68, p. 248, MRID 49547201.

[b] Fetal (litter) incidence

TABLE 30b. Visceral examinations, F_2 offspring from Cohort 1a [a]

Observations	Dose (ppm)			
	0	250	1000	2000
	Dose in mg/kg/day (M/F)			
	0/0	13.9/16.2	53.2/67.6	106.7/136.8
No. Fetuses(litters) examined	98 (20) [b]	111 (21)	90 (18)	106 (19)
Malformations/variations				
Major vessels- left-sided umbilical artery, anomalous pattern of major arteries	8 (5)	8 (7)	8 (4)	15 (9)
Urinary -abnormal consistency of the kidney, dilated ureter	2 (2)	0 (0)	1 (1)	0 (0)
Brain- dilated ventricles of the brain	0 (0)	1 (1)	0 (0)	0 (0)

[a] Data obtained from Table 69, pp. 249-251, MRID 49547201.

[b] Fetal (litter) incidence

TABLE 30c. Skeletal examinations, F_2 offspring from Cohort 1a [a]

Observations	Dose (ppm)			
	0	250	1000	2000
	Dose in mg/kg/day			
	0	16.2	67.6	136.8
No. Fetuses(litters) examined	112 (20)[b]	123 (21)	101 (18)	115 (19)
Malformations/variations				
Caudal arches-unossified and/or incompletely ossified	100 (20)	117 (21)	94 (18)	110 (19)
Unossified caudal arches with cartilage present % of affected litters/litters examined (mean % affected fetuses/litter)[c]	 70 34.9	 90 60.9	 78 66.3*	 100 68.5*

[a] Data obtained from Table 70, pp. 252-274, MRID 49547201.

[b] Fetal (litter) incidence

[c] Data presented in study report as % of affected fetuses/litter instead of fetal/litter incidence. Litter incidence (% affected/% examined) was calculated by the reviewer (not analyzed statistically).

* Statistically different ($p < 0.05$) from the control.

D. F_1 COHORT 1B REPRODUCTIVE TOXICITY WITH BREEDING:

1. **Mortality and clinical observations:** No treatment-related mortality or clinical signs were observed.

2. **Body weight:** Selected body weight data are given in Table 31. No changes were observed in body weight, body weight gain, or food consumption in females. The body weights of males in the high-dose group were decreased (7-10%) on PNDs 24 and 27, with statistical significance achieved on PND 27. The decreases are consistent with the decreases observed prior to weaning and are considered adverse due to the young age of the post-weaning animals. However, body weights of males showed recovery at later time points after weaning. As noted for Cohort 1a, the transient decrease beginning during late lactation may have been related to greater exposure to test material either from lactation or introduction to solid food. Food consumption was significantly increased by 7-9% for all treated males from study days 98-105, but the increases did not alter body weights or body weight gain.

TABLE 31. Mean body weight, body weight gain, and food consumption for F_1 Cohort 1b animals [ab]

Interval	Dose (ppm)			
	0	250	1000	2000
F_1 Cohort 1b males				
Dose in mg/kg/day				
	0	**13.9**	**53.2**	**106.7**
Body weight (g)				
PND 24	63.4±1.5	62.5±1.7	63.0±1.5	59.0±1.4 (-6.9)[c]
PND 27	81.1±1.9	79.0±2.0	79.2±1.7	73.4±2.4* (-9.5)
Day 0 [d]	146.3±3.50	148.7±3.07	148.2±3.72	143.1±2.52
Day 70	405.9±6.99	410.7±7.07	402.9±8.00	387.3±6.24
Day 105	450.6±8.78	457.3±8.39	445.3±9.16	429.1±6.57
Day 112	455.5±8.91	463.7±8.73	452.0±9.10	434.5±6.46
Day 140	488.8±29.95	469.9±25.66	488.6±29.36	483.9±6.62
Body weight gain (g)				
Day 0-140 [e]	342.5	321.2	340.4	340.8
Food consumption (g/kg/day)				
Days 0-7	143.0±5.16	147.2±4.69	145.0±3.96	149.4±2.37
Days 70-77	54.9±1.63	59.3±2.20	57.1±1.82	61.5±2.16
Days 98-105	48.7±0.58	52.4±1.29* (7.6)	53.1±1.16** (9.0)	54.0±1.01** (11)
Days 133.140	44.7±1.25	50.2±1.83	48.6±3.42	50.2±1.19
F_1 Cohort 1b females				
Dose in mg/kg/day				
	0	**16.2**	**67.6**	**136.8**
Body weight (g)				
PND 24	57.7±1.9	57.3±1.5	57.0±1.4	55.6±1.8
PND 27	71.0±1.6	72.9±1.7	70.4±1.4	69.3±1.8
Day 0 [d]	118.8±2.26	117.0±2.48	116.7±2.75	113.9±2.45
Day 35	194.2±3.16	191.0±3.64	196.1±2.67	192.6±3.23
Day 56	206.7±3.39	208.0±3.85	214.1±2.59	211.4±3.16
GD 0	214.8±3.93	212.6±3.89	218.2±2.42	215.4±3.23
GD 20	305.7±5.09	299.3±4.81	306.5±3.24	313.6±4.81
LD 0	241.2±4.38	240.9±5.00	244.0±4.04	245.1±3.88
LD 21	260.6±4.64	257.7±4.61	261.0±3.94	262.0±4.71
Body weight gain (g)				
Day 0-56 [e]	87.9	91	124.8	97.6
GD 0-20	90.9±3.44	86.7±2.97	88.3±2.47	98.2±3.76
Food consumption (g/kg/day)				
Days 0-7	135.0±3.34	131.0±2.81	141.7±4.06	141.2±3.16
Days 49-66	71.3±1.12	68.3±1.20	71.0±1.13	69.9±0.87
GD 0-6	79.0±3.01	72.3±2.66	77.3±3.23	77.4±1.34
GD 13-20	78.6±1.69	77.8±2.15	75.8±2.04	77.9±1.66
LD 0-4	108.0±4.31	104.1±5.46	107.9±5.38	109.6±5.21
LD 14-21	232.8±8.34	220.4±7.71	225.0±11.59	223.8±6.24

[a] Data obtained from Table 38 (pp. 204), Tables 77-78 (pp. 283-289), Tables 85-86 (pp. 300-301), and Tables 90-91 (pp. 305-306), MRID 49547201.

[b] Values are given as Mean ± Standard Deviation, n=14-20 for all groups except on Day 140, n=3/group.

[c] Numbers in parentheses equal percent change, relative to control value, calculated by reviewer.

[d] Animals were PND 34-40 on Day 0.

[e] Calculated by reviewer; not analyzed statistically.

* Statistically different ($p < 0.05$) from the control.

3. Clinical pathology:

a. Hematology: Selected hematology data are shown in Table 32. No hematology changes were observed in males. In females of the high-dose group, statistically significant increases in white blood cell counts and segmented absolute neutrophil counts were

observed that were outside of the historical control range. The increases were not considered adverse: for the segmented neutrophils, the values of all groups including controls were greater than the historical controls, and for the white blood cell count, low and mid dose counts were also above the historical values. Furthermore these changes were not seen in the parental animals. Increases in absolute lymphocyte counts (46%) and mean cell hemoglobin values (5%) were also observed in females, but were within the historical control range. In females of the mid-dose group, mean cell hemoglobin was increased by 7%, but the value was within historical control range.

Table 32. Selected Hematology Parameters in F_1 Offspring Cohort 1b[a]

Parameter	Dietary dose (ppm)			
	0	**250**	**1000**	**2000**
Males, PND 185				
Dose in mg/kg/day				
	0	**13.9**	**53.2**	**106.7**
Hematocrit (%)	49.1±1.8	47.6±2.8	47.9±1.6	47.8±1.2
Hemoglobin (g/dL)	16.0±1.6	15.7±1.8	15.9±0.4	16.0±0.3
RBC (10^6/mm^3)	9.27±0.37	9.13±0.58	9.15±0.49	9.29±0.42
MCH (pg)	17.3±0.6	17.2±0.6	17.4±0.7	17.2±0.8
WBC (10^6/mm^3)	5.5±0.9	6.4±1.6	6.5±1.2	6.5±1.0
Abs. lymphocyte (10^3/mm^3)	3.97±0.88	4.27±0.83	4.82±0.98	4.94±0.9
Abs. seg neutrophil (10^3/mm^3)	1.28±0.35	1.75±0.79	1.27±0.37	1.22±0.23
Females, LD 22				
Dose in mg/kg/day				
	0	**16.2**	**67.6**	**136.8**
Hematocrit (%)	49.8±2.4	49.7±1.7	50.4±3.4	51.0±1.7
Hemoglobin (g/dL)	17.2±0.7	17.0±0.3	16.8±0.9	17.1±0.5
RBC (10^6/mm^3)	8.57±0.47	8.77±0.47	9.0±0.48	8.98±0.38
MCH (pg)	20.0±0.4	19.4±0.7	18.7±0.5*(-7*)	19.0±0.6*(-5%)
WBC (10^6/mm^3)	7.8±0.7	9.0±1.7	8.3±1.2	11.0±1.2*(+40%)
Abs lymphocyte (10^3/mm^3)	3.58±1.05	4.45±0.67	4.44±0.21	5.30±0.98*(+46%)
Abs seg neutrophil (10^3/mm^3)	3.67±1.01	3.94±1.18	3.39±1.21	4.94±0.98*(+35%)

[a] Data obtained from Appendix L Tables 4-5 pp. 1678-1683, MRID 49547201.

[b] Values are given as Mean ± Standard Deviation, n=5-8 for all groups.

[c] Numbers in parentheses equal percent change, relative to control value, calculated by reviewer.

* Statistically different ($p < 0.05$) from the control.

b. <u>Clinical chemistry</u>: Selected clinical chemistry parameters are shown in Table 33. Alkaline phosphatase activity was significantly decreased in males of all dose groups (24-29%), but the values were within the historical control range. Triglyceride levels were significantly decreased in females of the mid- (49%) and high-dose (41%) groups, which is not considered an adverse effect.

Table 33. Selected clinical chemistry parameters – F_1 offspring Cohort 1b[a]				
Parameter evaluated (units)	**Dietary dose (ppm)**			
	0	**250**	**1000**	**2000**
Males (N = 8), PND 175				
Dose in mg/kg/day				
	0	**13.9**	**53.2**	**106.7**
Glucose (mg/dL)	123±36	137±17	124±15	126±20
Total cholesterol (mg/dL)	63±9	62±6	64±11	63±10
Triglycerides (mg/dL)	88±30	105±43	81±45	76±34
Urea (mg/dL)	12±1	12±1	12±2	11±2
Total protein (g/dL)	6.2±0.2	5.8±0.4	5.7±0.7	5.6±0.3
Albumin (g/dL)	3.4±0.1	3.1±0.3	3.1±0.3	3.2±0.1
AST (U/L)	72±13	75±14	64±15	60±10 (-17%)
ALT (U/L)	31±5	29±4	28±4	26±4
ALP (U/L)	86±17	65±13*(-24%)	64±11*(-26%)	61±19 (-29%)
Females (N = 5-7), PND 22				
Dose in mg/kg/day				
	0	**16.2**	**67.6**	**136.8**
Glucose (mg/dL)	100±11	112±18	106±32	113±30
Total cholesterol (mg/dL)	61±11	61±12	64±16	64±14
Triglycerides (mg/dL)	152±58	101±23	78±45*(-49%)	90±32* (-41%)
Urea (mg/dL)	22±3	23±5	23±2	28±7 (+23%)
Total protein (g/dL)	5.5±0.3	5.4±0.3	5.6±0.3	5.4±0.4
Albumin (g/dL)	3.2±0.2	3.2±0.1	3.3±0.2	3.2±0.2
AST (U/L)	111±38	93±11	94±14	99±6
ALT (U/L)	45±7	42±3	46±11	55±18
ALP (U/L)	75±21	104±48	102±60	103±34

a Data extracted from Table 8 of pathology report (Appendix L), pp. 1690 to 1693 of MRID 49547201.
Values in parentheses indicate % change compared to controls.
* Statistically significant, $p<0.05$

c. Urinalysis: There were no treatment-related effects on urine parameters in either sex.

d. Hormones: Thyroid hormone data are presented in Table 34. Testosterone (males), T4, and TSH levels (both sexes) were not significantly altered by treatment. In males of the mid- and high-dose groups, T3 levels were significantly decreased. There were no significant correlations observed between follicle cell height scores and thyroid hormones. T3 was not evaluated in females.

TABLE 34. Hormone data in F_1 Cohort 1b animals[ab]

Observation	Dose (ppm)			
	0	250	1000	2000
	Dose in mg/kg bw/day (M/F)			
	0/0	13.9/16.2	53.2/67.6	106.7/136.8
Males (PND 175)				
Testosterone (ng/dL)	53±9	54±11	52±11	42±12
T4 (µg/dL)	3.99±1.07	4.88±2.30	5.11±1.32	4.66±0.58
TSH (ng/mL)	1.44±0.57	1.72±0.98	1.63±0.73	1.51±0.80
T3 (ng/mL)	59.00±9.07	53.05±8.15	47.56±9.12* (-19.4)[c]	48.51±9.97* (-17.8)
Females (LD 22)				
T4 (µg/dL)	5.29±1.04	6.19±1.16	6.47±1.44	6.28±1.54
TSH (ng/mL)	5.00±2.58	3.36±1.86	3.77±1.03	4.81±1.91

[a] Data obtained from Appendix L Text Table G, p. 1643, Table 14 (p. 1722), MRID 49547201.

[b] Values are given as Mean ± Standard Deviation, n=10-12 for all groups.

[c] Numbers in parentheses equal percent change, relative to control value, calculated by reviewer.

* Statistically different ($p < 0.05$) from the control.

4. F_1 parental postmortem results:

a. Organ weights: Selected absolute and relative (to body) organ weight data from F_1 Cohort 1b males (PND 175) and females (LD 22) are shown in Table 35. Absolute and relative thyroid weights in females of the high-dose group were significantly increased, although there were no significant differences in thyroid colloid area and follicular cell height and thyroid hormone levels for this group. It is also noted that thyroids were weighed in grams instead of milligrams, which may have affected the magnitude reported due to rounding. Males did not show a similar increase. Other organ weight differences were observed but were not considered related to treatment due to low magnitude of change and/or lack of a dose-response relationship.

Table 35. Absolute and relative organ weights for F_1 offspring Cohort 1b[a]				
	Dietary dose (ppm)			
Organ weight (g)	**0**	**250**	**1000**	**2000**
Males (n = 20), PND 175				
Dose in mg/kg/day				
	0	**13.9**	**53.2**	**106.7**
Terminal body weight (g)	476.5±43.5	484.0±39.7	471.6±44.1	451.9±38.0 (-5%)
Liver abs	13.373±1.643	14.852±2.183	15.193±2.424* (+14%)	13.855±1.539
rel	2.880±0.34	3.065±0.344	3.230±0.477* (+12%)	3.061±0.209
Testes				
Left abs	1.931±0.172	1.889±0.178	1.903±0.204	1.843±0.135
rel	0.408±0.051	0.393±0.024	0.406±0.047	0.410±0.044
Right abs	1.923±0.174	1.844±0.190	1.859±0.176	1.839±0.150
Rel	0.406±0.049	0.383±0.048	0.396±0.041	0.409±0.047
Seminal vesicles w/fluid				
abs	1.335±0.167	1.266±0.180	1.290±0.247	1.264±0.250
rel	0.281±0.034	0.264±0.049	0.275±0.055	0.281±0.057
Brain abs	2.113±0.086	2.078±0.101	2.101±0.105	2.054±0.089
Rel	0.446±0.039	0.432±0.045	0.449±0.045	0.456±0.028
Heart abs	1.248±0.154	1.259±0.126	1.231±0.121	1.279±0.134
Rel	0.262±0.026	0.261±0.025	0.263±0.026	0.283±0.027*(+8%)
Thymus abs	0.472±0.096	0.503±0.104	0.525±0.116	0.449±0.083
Rel	0.100±0.021	0.104±0.021	0.111±0.021	0.100±0.018
Thyroid				
Left abs	0.016±0.003	0.017±0.005	0.016±0.003	0.016±0.003
rel	0.003±0.001	0.003±0.001	0.003±0.001	0.004±0.001
Right abs	0.019±0.005	0.020±0.006	0.018±0.005	0.018±0.003
rel	0.004±0.001	0.004±0.001	0.004±0.001	0.004±0.001
Females (n = 20), LD 22				
Dose in mg/kg/day				
	0	**16.2**	**67.6**	**136.8**
Terminal body weight (g)	253.2±20.4	255.6±29.5	256.4±25.5	260.2±29.0
Liver abs	12.001±2.339	12.145 ±2.475	12.884±2.640	13.288±2.700
rel	4.728±0.751	4.718±0.558	4.993±0.709	5.074±0.617
Brain abs	1.925±0.082	1.899±0.074	1.876±0.10	1.846±0.081* (-9%)
rel	0.764±0.052	0.752±0.091	0.737±0.063	0.621±0.170
Heart abs	0.985±0.132	0.976±0.119	1.002±0.107	1.005±0.084
rel	0.392±0.062	0.387±0.063	0.394±0.052	0.389±0.043
Thymus abs	0.228±0.065	0.255±0.079	0.262±0.085	0.251±0.069
rel	0.091±0.027	0.100±0.031	0.102±0.033	0.097±0.029
Thyroid				
Left abs	0.011±0.002	0.011±0.002	0.012±0.003	0.013±0.002*(+18%)
rel	0.004±0.001	0.004±0.001	0.005±0.001	0.005±0.001*(+25%)
Right abs	0.011±0.002	0.011±0.003	0.012±0.002	0.013±0.003 (+18%)
rel	0.004±0.001	0.004±0.001	0.005±0.001	0.005±0.001 (+25%)

a Data extracted from Tables 30-31 and 47-48 of pathology report (Appendix L), pp. 1748-1754, 1788-1794 of MRID 49547201. [b] Values are given as Mean ± Standard Deviation; n= 19-20 for all groups.

[c] Numbers in parentheses equal percent change, relative to control value, calculated by reviewer.

* Statistically different ($p < 0.05$) from the control.

b. <u>Pathology</u>:

1. **<u>Macroscopic examination</u>:** There were no treatment-related gross findings in 1b males or females. Dilatation of the kidney was observed at low incidence in all treated dose groups but did not show a dose-response (see Table 26, above).

2. **Microscopic examination:** No microscopic findings related to treatment were observed in the reproductive organs of male and females. A significant increase in incidence of dilatation of the renal pelvis was observed in males of the all treated groups when compared with controls. The incidence was 1/11, 3/3, 5/5, and 6/11 in the control, low-, mid-, and high-dose groups, respectively. A dose-response cannot be determined for the low and mid dose since only a few animals were examined. No calculi or test substance crystals or renal parenchyma damage were associated with the changes.

5. Reproductive parameters:

a. Cohort 1b estrous cycle: Data are shown in Table 36. There were no differences in the interval between vaginal opening and first estrus, mean estrous cycle length, or estrous cycle pattern among the groups.

TABLE 36. Estrous cycle data F_1 Cohort 1b parental females[ab]

Estrous cycle (days)	Dose (ppm)			
	0	250	1000	2000
	Dose in mg/kg/day			
	0	16.2	67.6	136.8
Mean length	5.2±0.3	4.9±0.2	5.6±0.5	4.9±0.1
Mean number of cycles	2.8±0.2	3.0±0.2	2.7±0.2	3.2±0.1

[a] Data obtained from Table 83, p. 298, MRID 49547201.

[b] Values are given as Mean ± Standard Error, n=18-20.

b. Cohort 1b sperm parameters: Data are shown in Table 37. There were no treatment-related effects on sperm motility, epididymal sperm counts, or sperm morphology. Testicular sperm counts in the mid- and high-dose groups were significantly increased by 43 and 46%, respectively, compared to control, but the effect is not considered adverse.

TABLE 37. Sperm data for F_1 Cohort 1b parental males[ab]				
Parameter	Dose (ppm)			
	0	250	1000	2000
	Dose in mg/kg/day			
	0	13.9	53.2	106.7
% Motile sperm	96.8±1.9	97.3±2.5	97.3±3.0	97.2±1.7
Testis sperm count (sperm/g)	25.4±6.7	31.4±7.8	36.3±9.4* (42.9)[c]	37.0±9.1* (45.7)
Epididymis sperm count (sperm/g)	183.7±59.1	175.7±46.5	162.4±34.4	161.1±60.8
Mean normal sperm	198.2±1.5	-	-	198.2±2.7
Mean abnormal sperm	1.7±1.3	-	-	1.5±2.8
Mean detached head sperm	0.1±0.3	-	-	0.3±0.5

[a] Data obtained from Text Table 30 (p. 98) and Table 94 (p. 309), MRID 49547201.

[b] Values are given as Mean ± Standard Deviation, n=9-10

[c] Numbers in parentheses equal percent change, relative to control value, calculated by reviewer.

* Statistically different ($p < 0.05$) from the control.

\- Not analyzed

c. <u>Cohort 1b reproductive performance</u>: The reproductive performances of the F_1 parental animals are summarized in Table 38. Ovarian follicular counts were similar between the control and high-dose group. There were no treatment-related effects on reproductive indices, number or corpora lutea or implantation, or sex ratio.

TABLE 38. Reproductive performance of F_1 Cohort 1b parental animals[ab]				
Observation	Dose (ppm)			
	0	250	1000	2000
	Dose in mg/kg/day (M/F)			
	0/0	13.9/16.2	53.2/67.6	106.7/136.8
Number of pregnancies	19	19	18	18
Mating index (%)	100	100	100	100
Fertility index (%)	100	95	90	100
Gestation index (%)	95	100	100	90
Gestation length (days) Range of days	22.1±0.08 22-23	22.1±0.07 22-23	22.1±0.06 22-23	22.2±0.14 21-24
Precoital interval (days) Range of days	2.3±0.24 1-4	2.7±0.27 1-6	2.4±0.20 1-4	2.5±0.21 1-4
Total No. implantations Implantations/Dam	199 9.9±0.64	181 9.5±0.49	165 9.2±0.72	202 10.1±0.75
Ovarian primordial follicles	35.4±7.55	-	-	34.7±9.40
Ovarian antral follicle	18.3±4.16	-	-	19.25±3.49
Corpora lutea	19.4±5.10	-	-	18.9±6.35

[a] Data obtained from Text Table 33 (p. 101) and Table 82 (p. 297), MRID 49547201.

[b] Values are given as Mean ± Error or Deviation

\- Not analyzed

F. <u>OFFSPRING</u>: F_2 (COHORT 1B)

1. <u>Viability and clinical signs</u>: Litter parameters for the F_2 offspring are summarized in Table 39. There were no treatment-related effects on the number of pup born, number of stillborn pups, sex ratio, anogenital distance, or viability indices. There were no treatment-related clinical observations in F_2 offspring.

TABLE 39. Litter parameters for Cohort 1b F_2 offspring [ab]				
Observation	Dose (ppm)			
	0	250	1000	2000
	Dose in mg/kg/day (M/F)			
	0/0	13.9/16.2	53.2/67.6	106.7/136.8
Number of litters	19	19	18	19
Number of pups born: Total	189	173	158	190
Stillborn	0	0	0	2
Found dead	0	1 (1)[c]	4 (1)	2 (2)
Mean litter size: PND 0	9.9±0.53	9.1±0.53	8.8±0.76	10.0±0.73
Mean number of live pups: PND 0	10	9	9	10
PND 4	10	9	9	10
PND 4 (post cull)	9	9	8	9
PND 21	9	8	8	9
Anogenital distance- cohort males (mm)	4.00±0.06	4.06±0.08	3.95±0.07	3.96±0.05
Anogenital distance- cohort females (mm)	2.18±0.04	2.20±0.04	2.28±0.05	2.36±0.11
Sex ratio (%male): PND 0	39.5±3.45	45.6±5.42	47.4±2.88	51.3±4.18
Birth index (%)	90.3±5.02	95.1±1.95	94.2±2.54	92.8±3.30
Live birth index (%)	100±0.00	100±0.00	100±0.00	93.6±5.33
Viability index (%)	99.5±0.53	99.4±0.58	91.8±5.78	97.2±1.95
Lactation index (%)	98.9±1.05	99.4±0.58	100±0.00	100±0.00

[a] Data obtained from Table 95 (pp. 310-311) and Table 99 (p. 322), MRID 49547201.

[b] Values are given as Mean ± Error

[c] Values in parentheses represent number of litters affected.

2. Body weight: Selected F_2 offspring body weight data are given in Tables 40 and 41.

a. F_2 offspring from birth to weaning: There were no treatment-related effects on body weight or body weight gains in male and female pups. Body weights were similar throughout the lactation period.

TABLE 40. Mean (±SE) Cohort 1b F_2 pup body weights (g) and body weight gain (g)[a]								
Observation Day	Dose (ppm)							
	0	250	1000	2000	0	250	1000	2000
	F_2 pups – male				F_2 pups – female			
	Dose in mg/kg/day							
	0	13.9	53.2	106.7	0	16.2	67.6	136.8
PND 0	6.2±0.10	6.3±0.10	6.2±0.14	6.1±0.12	5.9±0.08	5.9±0.08	5.8±0.10	5.8±0.11
PND 4[b]	10.6±0.24	10.9±0.22	10.5±0.27	10.4±0.33	10.3±0.22	10.4±0.17	10.0±0.22	10.0±0.27
PND 4[c]	10.6±0.24	10.9±0.22	10.5±0.27	10.4±0.33	10.3±0.22	10.4±0.17	10.0±0.22	10.0±0.26
PND 7	15.8±0.37	16.2±0.40	15.8±0.46	15.7±0.48	15.5±0.31	15.8±0.31	14.9±0.41	15.0±0.40
PND 14	28.2±0.86	30.1±0.90	29.0±0.91	28.7±0.98	27.6±0.76	29.2±0.83	27.9±0.89	27.4±0.87
PND 18	35.8±1.03	38.3±1.19	36.6±1.13	36.2±1.35	34.7±0.82	37.0±1.06	35.2±1.11	34.4±1.22
PND 21	45.4±1.15	49.1±1.39	47.1±1.18	45.7±1.71	44.0±0.89	46.6±1.13	45.0±1.16	43.3±1.47
Body weight gain								
PND 4-21	34.8±1.01	38.3±1.24	36.6±1.00	35.3±1.42	33.7±0.77	36.2±1.03	35.0±0.99	33.3±1.24
PND 0-21[d]	39.2	42.8	40.9	39.6	38.1	41.8	40.2	38.8

[a] Data from Tables 97-98, pp. 313-318, MRID 49547201. N = 17 – 19 litters

[b] Before culling.

[c] After (culling).

[d] Calculated by the reviewer. Not analyzed statistically.

b. F_2 females from weaning to PND 45: From PND 24 through PND 28 body weight of females in the high-dose group was significantly decreased compared to controls. The study author did not consider the decreases to be treatment-related due to two females in the high-dose groups with lower body weights. When the two females are not used in calculations, the mean body weights of females in the high-dose group are within 5% of control means (calculated by reviewer, data not shown). However, because of the young age of the post-weaning animals and similar findings of weight decreases were observed in F_1 offspring during the same early post-weaning period, the decrease was considered adverse. The pups did show recovery of body weight with time. Body weight gain (calculated by reviewer) was consistent among the groups. Food consumption was not different among the groups.

TABLE 41. Mean body weight and food consumption for F_2 females [ab]

Observations	Dose (ppm)			
	0	250	1000	2000
	Dose in mg/kg/day			
	0	16.2	67.6	136.8
Body weight (g)				
PND 24	58.5±1.3	58.9±0.9	57.7±1.3	53.0±1.8* (-9.4)[c]
PND 27	74.5±1.5	73.9±1.2	71.9±1.7	66.6±2.1** (-10.6)
PND 28	78.6±1.4	78.6±1.3	77.1±1.8	72.5±2.0* (-7.8)
PND 35	113.8±1.6	111.7±1.6	112.1±2.4	107.6±2.5
PND 42	127.2±1.5	125.9±1.6	125.9±2.7	124.3±2.2
PND 45	147.0±4.7	146.6±5.8	145.4±5.2	137.9±6.5
Body weight gain (g)[d]				
PND 24-45	88.5	87.7	87.7	84.9
Food consumption g/animal/day)				
PND 28-35	15.6±0.3	14.7±0.4	14.7±0.3	15.3±0.4
PND 35-42	18.1±0.4	18.1±0.9	17.4±0.4	17.9±0.5
PND 42-44	17.4±0.7	16.9±1.6	16.1±0.5	16.6±0.8

[a] Data obtained from Tables 101 and 103, pp. 324 and 326, MRID 49547201.

[b] Values are given as Mean ± Standard Error; n= 18-20 females for all groups except on PND 42-45, n= 4-7/group.

[c] Numbers in parentheses equal percent change, relative to control value, calculated by reviewer.

[d] Calculated by reviewer. Not analyzed statistically.

* Statistically different ($p < 0.05$) from the control.

** Statistically different ($p < 0.01$) from the control.

3. **Developmental landmarks:** There were no treatment-related effects on nipple/areola retention among the groups.

4. **Sexual maturity:** The day of vaginal opening was similar among the F_2 groups (Table 42). The mean day of vaginal opening was 30.6, 31.6, 30.2, and 31.9 for the control, low-, mid-, and high-dose groups, respectively. At the time of necropsy on PND 45, some females in the low- (2), mid- (1), and high-dose (2) groups had not reached vaginal patency.

Table 42. Sexual maturation of F_2 cohort 1b offspring females[a]				
Vaginal opening	**Dietary dose (ppm)**			
	0	**250**	**1000**	**2000**
	Dose in mg/kg/day			
	0	**16.2**	**67.6**	**136.8**
Age (days)	30.6±2.9	31.6±5.2	30.2±2.6	31.9±3.8 (+1.3)
Body weight (g)	93.8±14.3	93.5±21.9	87.3±17.6	92.5±20.5

[a] Data extracted from Table 104, p. 327 of MRID 49547201. n = 18 – 20.

5. **Hormones:** Thyroid hormone data are presented in Table 43. Increases (not significant) in T4 (20%) in males and TSH (26%) in females were observed at PND 23. There were no significant effects on colloid area or follicular cell height associated with these changes in either sex, but the finding is considered a possible effect of treatment since cohort 3 PND 23 pups showed thyroid effects. Females at PND 45 showed an increase in T4 (52%) and TSH (41%) along with semi-quantitative changes (Table 46, below); therefore the findings were considered to be treatment-related.

TABLE 43. Thyroid hormone data in F_2 animals[ab]				
Observation	**Dose (ppm)**			
	0	**250**	**1000**	**2000**
	Dose in mg/kg bw/day (M/F)			
	0/0	**13.9/16.2**	**53.2/67.6**	**106.7/136.8**
Males (PND 23)				
T4 (µg/dL)	3.60±1.06	3.61±0.90	3.87±0.99	4.32±0.69 (+20)
TSH (ng/mL)	0.76±0.38	0.88±0.26	0.94±0.19	0.81±0.17
Females (PND 23 and 45)				
PND 23 T4 (µg/dL)	4.37±0.86	4.30±0.99	3.90±0.82	4.53±1.11
PND 23 TSH (ng/mL)	0.74±0.24	0.88±0.26	0.89±0.19	0.93±0.25 (+26)
PND 45 T4 (µg/dL)	3.74±1.23	4.44±0.87	4.46±1.26	5.70±2.04* (+52.4)[c]
PND 45 TSH (ng/mL)	0.88±0.17	1.15±0.33	1.12±0.25	1.24±0.54 (+40.9)

[a] Data obtained from Text Table 36 (p. 105) and Appendix L Text Table G (p. 1643), MRID 49547201.

[b] Values are given as Mean ± Standard Deviation, n=12 for all groups.

[c] Numbers in parentheses equal percent change, relative to control value.

* Statistically different ($p < 0.05$) from the control.

6. **Offspring postmortem results:**

   a. **Organ weights:** Selected organ weight data from F_2 offspring are shown in Table 44. There were no treatment-related effects on absolute or relative organ weights from F_2 animals sacrificed on PNDs 21 and 45. Relative liver weights in males of the mid- and high-dose groups were significantly increased, 8 and 13%, respectively, at PND 23. Absolute brain weights from females in the high-dose group were significantly decreased by 4%, and relative pituitary weights were increased by 33% on PND 23. The differences were not considered adverse due to the small magnitude of the changes. There were no gross or histopathological findings correlating with the changes in organ weights.

Table 44. Absolute and relative organ weights for F_2 offspring from cohort 1b[a]				
	Dietary dose (ppm)			
Organ weight (g)	**0**	**250**	**1000**	**2000**
Males (N = 12), PND 23				
Dose in mg/kg/day				
	0	**13.9**	**53.2**	**106.7**
Terminal body weight (g)	51.8±8.6	58.2±8.5	54.4±5.8	53.8±8.7
Liver abs	2.102±0.406	2.413±0.435 (+15%)	2.381±0.236 (+13%)	2.457±0.434 (+17%)
rel	4.040±0.255	4.134±0.253	4.383±0.211* (+8%)	4.564±0.226*(+13%)
Testes				
Left abs	0.0161±0.030	0.180±0.036	0.164±0.015	0.155±0.026
rel	0.311±0.028	0.309±0.030	0.302±0.023	0.289±0.017
Right abs	0.161±0.025	0.177±0.034	0.166±0.013	0.157±0.027
rel	0.313±0.027	0.303±0.031	0.306±0.024	0.292±0.019
Brain abs	1.501±0.08	1.494±0.068	1.479±0.069	1.461±0.079
rel	2.978±0.571	2.605±0.289	2.737±0.212	2.755±0.273
Thymus abs	0.191±0.039	0.214±0.031	0.206±0.034	0.194±0.036
rel	0.369±0.044	0.369±0.039	0.377±0.042	0.360±0.040
Thyroid (left + right)				
abs	0.007±0.002	0.006±0.001	0.007±0.002	0.006±0.001
rel	0.0130±0.034	0.0105±0.0022	0.0125±0.0028	0.0117±0.0029
Pituitary abs	0.003±0.001	0.003±0.001	0.003±0.001	0.003±0.001
rel	0.0057±0.0029	0.0053±0.0014	0.0051±0.0017	0.0053±0.0013
Females (N = 12), PND 23				
Dose in mg/kg/day				
	0	**16.2**	**67.6**	**136.8**
Terminal body weight (g)	52.6±3.7	55.0±5.8	51.8±5.5	49.4±9.5 (-6%)
Liver abs	2.287±0.230	2.376 ±0.315	2.250±0.285	2.228±0.487
rel	4.347±0.241	4.317±0.243	4.340±0.206	4.494±0.299
Brain abs	1.459±0.055	1.472±0.059	1.421±0.051 (-3%)	1.397±0.059* (-4%)
rel	2.787±0.186	2.701±0.249	2.769±0.268	2.939±0.683 (+5%)
Thymus abs	0.206±0.027	0.221±0.037	0.199±0.027	0.186±0.048
rel	0.391±0.041	0.401±0.045	0.386±0.055	0.372±0.051
Thyroid (left + right)				
abs	0.006±0.001	0.006±0.001	0.007±0.002	0.006±0.002
rel	0.0107±0.0025	0.0105±0.0022	0.0127±0.0040	0.0119±0.0037
Pituitary abs	0.003±0.001	0.003±0.001	0.003±0.001	0.003±0.001
rel	0.0054±0.0013	0.0051±0.0011	0.0061±0.0014 (+13%)	0.0072±0.0029* (+33%)

[a] Data extracted from Tables 40-41 and 51-52 of pathology report (Appendix L), pp. 1765-1770 and 1800-1805 of MRID 49547201. Values in parentheses indicate percent change relative to controls.
* statistically significant, $p<0.05$

b. Pathology:

1. **Macroscopic examination:** There were no treatment-related gross findings in males or F_2 offspring.

2. **Microscopic examination:** No treatment-related abnormalities of the thyroid gland were observed in F_2 males and females on PND 23 or in females on PND 45 (see Section G, below, for details). There were no treatment-related effects on brain measurements on PND 21 (see Table 51, below).

G. STATISTICAL ANALYSIS OF THYROID SEMI-QUANTITATIVE MICROSCOPIC SCORING AND HORMONE DATA

1. **P parental animals:** Results of the statistical analysis for colloid area and follicular cell height are presented below in Table 45. In females at 1000 and 2000 ppm, statistically significant increases in follicular cell height (+26%, both doses) and decrease in colloid area (-14% and -13%, respectively) were observed. No significant increases or decreases were observed in males at any dose.

Table 45. Parental P animals semi-quantitative microscopic scoring of thyroid gland follicular cells[1]

Parameter	Dietary dose (ppm)			
	0	250	1000	2000
	Dose in mg/kg bw/day (M/F)			
	0/0	13.9/16.2	53.2/67.6	106.7/136.8
Males (n = 23-30)				
Colloid area	4.421±0.1281	4.250±0.1230	3.842±0.1210	4.391±0.1189
Follicle height	1.483±0.1232	1.786±0.1181	2.072±0.1162	1.590±0.1142
Females (n = 18-27)				
Colloid area	3.754±0.1466	3.685±0.1409	3.231±0.1432* (-14%)	3.265±0.1636*(-13%)
Follicle height	2.185±0.1451	2.298±0.01395	2.750±0.1418 (+26%)	2.749±0.1620* (+26%)

1 Data extracted from Tables 3.1 and 3.2 (Appendix D of the pathology report in study report Appendix L), pp. 3603-3622 of MRID 49547201. Values in parentheses indicate percent change relative to controls.
* statistically significant, $p<0.05$.

No positive association of follicular cell height with TSH was identified. The analysis of follicle cell height and T4 levels showed a positive association only in males ($p<0.017$) at 2000 ppm. No significant associations were observed for other treatment groups of males or females at any dose.

2. **F_1 Cohort 1a (males only), 1b and 3 animals:** Results of the statistical analysis for colloid area and follicular cell height are presented below in Table 46. In Cohort 3 (PND 23), males at 1000 and 2000 ppm showed statistically significant decreases in colloid area (-12% and -15%, respectively) and increases in follicular cell height (+43% and +55%, respectively). Females in the 2000 ppm dose groups of Cohorts 1b (LD 22) and 3 (PND 23) showed statistically significant decrease in colloid area (-23%, both groups) and increases in follicular cell height (+38% and +55%, respectively). No significant changes were observed in males of Cohorts 1a (PND 148) and 1b (PND 178).

Table 46. F_1 offspring cohort groups semi-quantitative microscopic scoring of thyroid gland follicular cells (presented as least square mean ± standard error)[1]

Parameter	Dietary dose (ppm)			
	0	250	1000	2000
	Dose in mg/kg bw/day (M/F)			
	0/0	13.9/16.2	53.2/67.6	106.7/136.8
Males (n = 19-22, Cohorts 1a, 1b; 10-12 Cohort 3)				
Cohort 1a (PND 148)				
Colloid area	4.364±0.1249	4.432±0.1249	4.295±0.1249	4.523±0.1249
Follicle height	1.682±0.1290	1.568±0.1290	1.659±0.1290	1.432±0.1290
Cohort 1b (PND 178)				
Colloid area	4.400±0.1473	4.477±0.1474	4.176±0.1474	4.600±0.1473
Follicle height	1.550±0.1465	1.473±0.1466	1.725±0.1466	1.300±0.1465
Cohort 3 (PND 23)				
Colloid area	4.917±0.1373	4.800±0.1505	4.328±0.1379*(-12%)	4.182±0.1432**(-15%)
Follicle height	1.167±0.1428	1.200±0.1565	1.671±0.1433*(+43%)	1.818±0.1492**(+55%)
Females (n = 17-19, Cohort 1b; 10-12, Cohort 3)				
Cohort 1b (LD 22)				
Colloid area	3.843±0.1846	3.786±0.1846	3.765±0.1951	2.941±0.1951*(-23%)
Follicle height	2.157±0.1818	2.1562±0.1818	2.235±0.1921	2.971±0.1921*(+38%)
Cohort 3 (PND 23)				
Colloid area	4.749±0.1402	4.500±0.1406	4.500±0.1461	3.682±0.146**(-22%)
Follicle height	1.251±0.1402	1.500±0.1406	1.500±0.1462	2.318±0.1461**(+85%)

1 Data extracted from Tables 3.1 and 3.2 (Appendix D of the pathology report in study report Appendix L), pp. 3603-3622 of MRID 49547201.
* statistically significant, $p<0.05$ ** statistically significant, $p<0.01$

No statistically significant positive association of follicular cell height with TSH was identified and a consistent pattern of association of follicular cell height and T4 was not observed. The only positive statistically significant association between follicular cell height and T4 was observed for F_1 Cohort 1b control females ($p<0.027$) and Cohort 3 control females ($p<0.011$). It is noted, however, that the PND 23 males showed an increase in TSH at 2000 ppm.

3. **F_2 offspring animals (PND 23 males and females, PND 45 females):** Results of the statistical analysis for colloid area and follicular cell height are presented below in Table 47. At 2000 ppm, females showed a statistically significant decrease in colloid area (-19%) and an increase in follicular cell height (+60%). There were no effects observed in either sex at PND 23.

Table 47. F_2 offspring animals semi-quantitative microscopic scoring of thyroid gland follicular cells[1]				
Parameter	Dietary dose (ppm)			
	0	250	1000	2000
	Dose in mg/kg/day (M/F)			
	0/0	13.9/16.2	53.2/67.6	106.7/136.8
Males (n = 11-12), PND 23				
Colloid area	5.000±0.0459	5.000±0.0455	4.875±0.0451	5.000±0.0451
Follicle height	1.000±0.04587	1.000±0.04553	1.125±0.0451	1.000±0.4514
Females (n = 12), PND 23				
Colloid area	5.000±0.0208	5.000±0.0208	4.958±0.0.208	5.000±0.0208
Follicle height	1.000±0.02083	1.000±0.002083	1.042±0.0208	1.000±0.0208
Females (n = 20), PND 45				
Colloid area	4.725±0.1417	4.700±0.1417	4.650±0.1417	3.850±0.1417** (-19%)
Follicle height	1.300±0.1436	1.300±0.1436	1.400±0.1436	2.075±0.1436* (+60%)

1 Data extracted from Tables 3.1 and 3.2 (Appendix D of the pathology report in study report Appendix L), pp. 3603-3622 of MRID 49547201. Values in parentheses indicate percent change relative to controls.
* statistically significant, $p<0.05$ ** statistically significant, $p<0.01$

Analysis of a statistically significant positive association between follicular cell height and thyroid hormone levels did not indicate a consistent pattern. No positive association of follicular cell height with TSH was identified. A statistically significant positive association of follicle cell height and T4 level was observed in PND 45 females at 250 ppm ($p<0.048$) and 2000 ppm ($p<0.046$), but not at 1000 ppm. It is noted that T4 and TSH were both increased in females at 2000 ppm.

H. F_1 COHORT 2A DEVELOPMENTAL NEUROTOXICITY:

1. **Mortality and clinical observations:** There were no treatment-related clinical signs or mortality.

2. **Body weight and food consumption:** Selected body weight data are given in Table 48. There were no treatment-related effects on body weight, body weight gain, or food consumption in either sex.

TABLE 48. Mean body weight, body weight gain, and food consumption for F_1 Cohort 2a males [ab]

Interval	Dose (ppm)			
	0	250	1000	2000
F_1 Cohort 2a males				
Dose in mg/kg/day				
	0	**13.9**	**53.2**	**106.7**
Body weight (g)				
PND 24	62.0±2.3	62.2±2.4	61.8±1.6	61.3±2.9
PND 28	84.5±2.8	84.5±2.6	85.6±2.2	82.2±3.9
PND 35	134.6±4.0	134.1±3.6	134.5±3.9	131.9±4.8
Day 0[c]	145.0±3.31	146.8±4.76	148.2±5.21	146.8±6.44
Day 14	239.4±5.35	233.5±6.39	234.9±9.01	237.4±9.99
Day 28	294.9±8.23	285.9±6.82	288.9±7.96	283.8±9.55
Body weight gain (g)				
PND 24-Day 28[d]	232.9	223.7	227.1	222.5
Food consumption (g/kg/day)				
PND 28-35	20.5±1.5	19.1±2.3	18.1±1.2	21.0±1.3
Days 0-7	139.8±3.41	139.7±8.88	133.0±4.90	140.3±4.33
Days 21-28	101.3±5.54	101.2±7.74	101.3±4.13	106.5±5.91
F_1 Cohort 2a females				
Dose in mg/kg/day				
	0	**16.2**	**67.6**	**136.8**
Body weight (g)				
PND 24	60.3±1.5	59.3±3.1	58.6±1.2	55.9±1.5
PND 28	79.8±1.7	77.9±3.7	76.9±1.4	74.6±1.9
PND 35	111.2±3.2	111.7±4.3	110.4±3.2	110.0±3.0
Day 0 [c]	116.8±3.37	117.7±3.70	113.8±3.06	113.5±3.19
Day 14	157.3±3.74	157.8±4.22	156.0±3.67	152.4±4.82
Day 28	182.5±4.74	183.6±4.42	184.2±4.91	180.6±4.43
Body weight gain (g)				
PND 24-Day 28[d]	122.2	124.3	125.6	124.7
Food consumption (g/kg/day)				
Days 28-35	21.0±1.7	18.1±1.8	22.8±2.7	20.7±1.7
Days 0-7	141.9±3.91	158.0±7.63	151.9±3.83	165.7±10.96
Days 21-28	98.3±3.24	99.1±1.95	97.6±1.44	105.3±2.85

[a] Data obtained from Table 39 (pp. 206-207), Table 43 (p. 211), and Tables 106-107 (pp. 330-333), MRID 49547201.

[b] Values are given as Mean ± Standard Error, n=10/group except Day 112, n=5/group.

[c] Data collected during weeks of PNDs 36, 50, and 64.

[d] Calculated by reviewer. Not analyzed statistically.

3. **Ophthalmology examination:** There were no treatment-related effects observed in either sex.

4. **Neurological evaluations/FOB findings:** No treatment-related effects were seen during the FOB testing.

   Startle amplitude and startle latency values for F_1 Cohort 2a offspring are presented in Table 49. No treatment-related effects were observed on either parameter. Habituation was observed in all groups: the degree of the habituation varied among the test groups, but did not show a dose-related effect.

Table 49. Auditory startle reflex peak amplitude (g) data by time block interval and average peak amplitude and latency to peak (msec) all blocks – F_1 Offspring Cohort 2A, PND 58-61[a]				
Time block # [b]	**Dose group (ppm)**			
	0	**250**	**1000**	**2000**
Males (n = 10)				
Dose in mg/kg/day				
	0	**13.9**	**53.2**	**106.7**
Body weight (g)	282	260	258	247 (↓13%)
1	317±103	252±101	304±109	206±82
2	284±82	225±100	257±137	266±122
3	291±122	195±87	208±131	255±112
4	219±71	171±88	164±117	189±94
5	193±91	164±99	152±105	167±90
Avg. peak amplitude blocks 1-5, g	261±79	202±82	217±111	217±89
Avg. latency to peak, msec	39±2	39±2	37±2	38±2
Females (n = 10)				
Dose in mg/kg/day				
	0	**16.2**	**67.6**	**136.8**
Body weight (g)	180	181	174	177
1	111±83	130±57	109±42	124±81
2	149±122	168±84	162±84	135±68
3	126±99	151±100	130±73	121±77
4	94±71	100±34	74±29	83±48
5	82±53	97±42	74±26	63±21
Avg. peak amplitude blocks 1-5, g	112±81	129±49	110±39	105±53
Avg. latency to peak, msec	40±5	38±3	39±2	38±2

[a] Data extracted from Table 115 , pp. 366-369 of MRID 49547201. Values are presented as SD±mean.
[b] Time block intervals values are averages of 10 trials/block

Total motor and locomotor activity data for F_1 Cohort 2a offspring are presented in Table 50 and 51, respectively). There were no treatment-related effects on motor and locomotor activity in males or females. Some groups, including controls, showed mean total counts either greater or lower than the historical controls from a similar PND age. Habituation over the course of measurement intervals was observed for all groups.

Table 50. Motor Activity (MA) Counts per Interval and Total MA Counts – Cohort 2A, PND 63-68 [a]

Dose[b] (ppm)	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Total	Historical control (PND 60)
Males (n = 10)								
0	123±37	110±39	103±35	97±41	83±30	83±23	599±124	485-571
250	121±16	115±34	125±41	100±29	82±17	75±30	616±123	
1000	114±24	96±36	95±29	85±27	87±34	88±44	565±118	
2000	117+25	91±17	104±31	82±24	89±18	83±22	565±100	
Females (n = 10)								
0	139±40	109±50	90±27	92±39	91±23	90±34	609±169	656-741
250	148±19	133±36	125±35	112±34	103±36	93±26	713±132	
1000	161±73	135±35	131±52	125±49	104±45	105±39	761±263	
2000	137±49	118±49	121±43	110±41	98±48	107±50	691±218	

[a] Data obtained from Table 111, pp. 358-359 and Table 113, pp. 362-363, MRID 49547201. Historical control data were obtained via personal communication with the registrant. Values presented as mean±SD.

[b] Doses (low to high) are equivalent to 13.9, 53.2 or 106.7 mg/kg/day in males and 16.2, 67.6 or 136.8 mg/kg/day in females.

Table 51. Locomotor Activity Counts per Interval and Total Locomotor Counts[a] – Cohort 2A, PND 63-68

Dose[b] (ppm)	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Total	Historical control
Males (n = 10)								
0	89±35	76±38	71±37	65±33	51±23	52±16	404±116	332-395
250	82±13	81±21	87±31	67±20	55±17	47±21	420±88	
1000	79±26	59±30	64±34	57±27	55±26	56±34	369±137	
2000	75±16	55±14	70±30	55±22	58±21	50±14	362±91	
Females (n = 10)								
0	90±24	67±31	54±19	56±21	55±22	56±26	378±96	425-494
250	94±10	87±29	85±34	73±29	62±29	49±24	450±114	
1000	96±30	83±24	86±32	80±32	61±28	63±24	468±136	
2000	92±33	76±34	76±36	73±32	58±32	70±40	445±145	

[a] Data obtained from Table 112, pp. 360-361 and Table 114, pp. 364-365 of MRID 49547201. Historical control data were obtained via personal communication with the registrant. Values presented as mean±SD.

[b] Doses (low to high) are equivalent to 13.9, 53.2 or 106.7 mg/kg/day in males and 16.2, 67.6 or 136.8 mg/kg/day in females.

5. Postmortem results:

a. Brain histomorphometry: Selected brain morphometry data from F_1 Cohort 2a animals on PND 70 are shown in Table 52. No statistically significant differences in brain weight were observed. In males of the low- and high-dose groups, the thickness of the frontal and parietal cortex was significantly decreased compared to controls. In males of the high-dose group, the width of the caudate putamen was also significantly decreased. In females, frontal and parietal cortex thickness was significantly decreased. The frontal cortex thickness of females in the mid-dose group was significantly decreased as well. The differences were attributed to random brain weight differences and were not considered adverse because of the small magnitude of the changes (generally ~3 to 6%),

lack of dose-response relationship for some measurements, and no correlating findings in nervous system histopathology, FOB, motor activity, or auditory startle parameters.

TABLE 52. Brain morphometry data in F_1 Cohort 2a animals [ab]

Observations	Dose (ppm)			
	0	250	1000	2000
Males				
Dose in mg/kg/day				
	0	**13.9**	**53.2**	**106.7**
Body weight (g)	318.4±28.8	312.5±19.4	314.9±31.4	310.3±34.1
Brain weight (g)	1.843±0.109	1.822±0.088	1.780±0.073	1.791±0.102
Cerebrum length A/P^2 (mm)	14.78±0.59	14.68±0.36	14.88±0.46	14.82±0.41
Cerebellum length A/P (mm)	8.08±0.54	8.07±0.28	8.38±0.17	8.33±0.66
Cerebellum height (mm)	4.1684±0.237	4.2161±0.135	4.1906±0.153	4.1884±0.095
Frontal cortex (mm)	1.3866±0.040	1.2955±0.053* (-6.6)[c]	1.3623±0.071	1.3456±0.042* (-2.9)
Parietal cortex (mm)	1.4919±0.073	1.3613±0.037* (-8.8)	1.4473±0.044	1.4101±0.056* (-5.5)
Caudate putamen (mm)	2.7714±0.108	2.7173±0.123	2.7244±0.099	2.6238±0.088* (-5.3)
Hippocampus (mm)	1.4256±0.103	1.3698±0.085	1.3275±0.086*	1.4293±0.075
Females				
Dose in mg/kg/day				
	0	**16.2**	**67.6**	**136.8**
Body weight (g)	188.7±14.6	191.5±14.3	192.2±14.1	187.0±15.4
Brain weight (g)	1.675±0.087	1.712±0.063	1.662±0.091	1.617±0.075
Cerebrum length A/P^2 (mm)	14.73±0.42	14.39±0.28	14.40±0.45	14.26±0.36
Cerebellum length A/P (mm)	8.22±0.43	8.17±0.16	8.3±0.35	8.14±0.51
Cerebellum height (mm)	4.144±0.169	4.0402±0.156	3.8972±0.250* (-6)	4.053±0.177
Frontal cortex (mm)	1.3443±0.041	1.3210±0.039	**1**.3061±0.043* (-2.8)	1.3008±0.050* (-3.2)
Parietal cortex (mm)	1.4124±0.042	1.3861±0.063	1.3799±0.053	1.3313±0.049* (-5.7)
Caudate putamen (mm)	2.6323±0.114	2.6489±0.140	2.4406±0.049* (-7.3)	2.5974±0.130
Hippocampus (mm)	1.3229±0.095	1.2910±0.076	1.3068±0.133	1.3509±0.060

[a] Data obtained from Appendix L Table 54, p. 1808, MRID 49547201.

[b] Values are given as Mean ± Standard Deviation; n= 10 for all groups.

[c] Numbers in parentheses equal percent change, relative to control value, calculated by reviewer.

* Statistically different ($p < 0.05$) from the control.

b. Pathology:

1. **Macroscopic examination:** There were no treatment-related gross findings in males or females.

2. **Microscopic examination:** Histopathologic examination of the perfused animals showed no treatment-related findings on the nervous system.

I. F_1 COHORT 2B DEVELOPMENTAL NEUROPATHOLOGY:

1. **Mortality and clinical observations:** There were no treatment-related clinical signs or mortality.

2. **F_1 Cohort 2b postmortem results:**

a. Brain weights: There were no statistically significant effects on final body or brain weights in males or females on PND 21. Brain weight and morphometrics data are

summarized in Table 53.

Table 53. Brain Weight and Morphometrics[1] – Cohort 2b PND 21[1]

Measurement	Dietary dose (ppm)			
	0	250	1000	2000
Males (n = 11-12)				
Dose in mg/kg/day				
	0	**13.9**	**53.2**	**106.7**
Body weight (g)	46.3±5.2	46.9±6.8	45.7±4.2	45.1±4.5
Brain weight (fixed) (g)	1.389±0.061	1.382±0.057	1.350±0.056	1.346±0.065
Cerebrum Length, A/P[2] (mm)	13.61±0.36	13.50±0.19	13.56±0.36	13.55±0.50
Cerebellum Length, A/P (mm)	7.49±0.34	7.47±0.39	7.56±0.24	7.38±0.39
Cerebrum Height (mm)	3.7667±0.261	--	--	3.8750±0.158
Frontal Cortex (mm)	1.2088±0.102	--	--	1.2563±0.062
Parietal Cortex (mm)	1.3172±0.116	--	--	1.3434±0.069
Caudate Putamen (mm)	2.2515±0.066	--	--	2.2747±0.088
Hippocampus	1.2380±0.101	--	--	1.1826±0.105
Females (n = 12-13)				
Dose in mg/kg/day				
	0	**16.2**	**67.6**	**136.8**
Body weight (g)	46.3±4.9	44.1±8.3	44.9±6.7	44.1±4.7
Brain weight (fixed) (g)	1.339±0.090	1.341±0.080	1.331±0.044	1.303±0.082
Cerebrum Length, A/P (mm)	13.47±0.44	13.38±0.41	13.51±0.30	13.17±0.47
Cerebellum Length, A/P (mm)	7.57±0.50	7.43±0.38	7.38±0.32	7.45±0.41
Cerebellum Height (mm)	3.7583±0.357	--	--	3.8294±0.251
Frontal Cortex (mm)	1.2678±0.095	--	--	1.2659±0.052
Parietal Cortex (mm)	1.3130±0.117	--	--	1.2924±0.065
Caudate Putamen (mm)	2.3200±0.144	--	--	2.522±0.138
Hippocampus (mm)	1.2165±0.118	--	--	1.1994±0.076

[1] Data extracted from Tables 36, p. 1761; 37, p. 1762 and 54, pp.1808-1809, of the pathology report (Appendix L of study report), MRID 49547201; only brains from the control and high dose groups were evaluated at the microscopic level. Shaded areas of table – not evaluated.

[2] A/P - anterior/posterior

b. Pathology:

1. **Macroscopic examination:** There were no treatment-related gross findings in males or females on PND 21.

2. **Microscopic examination:** There were no treatment-related histopathologic findings in the nervous system or structural changes in the brains of rats in F_1 Cohort 2b.

J. F_2 COHORT 1B BRAIN WEIGHT AND MORPHOMETRICS: Brain weight and selected morphometrics (gross measurements) are shown below in Table 54. No treatment-related effects were observed.

Table 54. Brain Weight and Gross Measurements[1] – Cohort 1b F_2 PND 21[1]

Measurement	Dietary dose (ppm)			
	0	250	1000	2000
Males (N = 12)				
Dose in mg/kg/day				
	0	**13.9**	**53.2**	**106.7**
Body weight (g)	44.9±3.5	45.6±6.1	46.7±5.1	44.3±8.0
Brain weight (fixed) (g)	1.362±0.091	1.345±0.057	1.367±0.043	1.347±0.089
Cerebrum Length A/P[2] (mm)	13.54±0.35	13.37±0.41	13.30±0.32	13.45±0.35
Cerebellum Length A/P (mm)	7.59±0.40	7.59±0.33	7.43±0.30	7.36±0.53
Females (N = 12)				
Dose in mg/kg/day				
	0	**16.2**	**67.6**	**136.8**
Body weight (g)	44.9±5.7	49.3±6.1	45.1±4.2	43.0±7.0
Brain weight (fixed) (g)	1.421±0.091	1.407±0.064	1.367±0.057	1.307±0.073
Cerebrum Length A/P (mm)	13.79±0.36	13.63±0.25	13.58±0.26	13.38±0.33
Cerebellum Length A/P (mm)	7.51±0.49	7.53±0.26	7.45±0.22	7.38±0.46

[1] Data obtained from pathology report Appendix A, pp. 2394-2398 of MRID 49547201.

2 A/P - Anterior/Posterior

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The investigators concluded that there were no effects of treatment for systemic toxicity of parental animals, reproductive toxicity, thyroid effects, developmental effects or sexual maturation or developmental neurotoxicity. Decreased pup weight at 2000 ppm was considered an adverse effects of treatment. Systemic toxicity was assessed across life stages. No mortalities or clinical signs attributable to treatment were observed. Decreased body weight was observed in F_1 males of the high-dose group and is considered adverse. Increased white blood cell counts and segmented absolute neutrophil counts were observed in F_1 females and are considered treatment-related but not adverse. Liver weight increases were observed across multiple life stages at 1000 and 2000 ppm, but were likely reflective of adaptive changes. Thymus weights were increased in F_1 Cohort 1a males and females, but no gross or histopathological correlates were observed in other Cohorts. There were no treatment-related effects on kidney, spleen, adrenal glands, heart, lungs, bone marrow, or lymph nodes.

There were no treatment-related effects on male reproduction parameters including mating index, male fertility, preputial separation, testosterone levels, sperm motility, epididymal sperm counts, sperm morphology, testis, seminal vesicles, epididymis, prostate, and LABC weights, or histology of all reproductive organs. Testicular sperm counts were significantly increased in F_1 Cohort 1b animals of the mid- and high-dose groups, but the increases were not considered adverse.

There were no treatment-related effects on female reproduction parameters including reproduction indices, duration of gestation, numbers of corpora lutea, implantation sites, ovarian follicles, vaginal opening, estrous cyclicity, and uterus and ovary weights and histopathology.

There were no treatment-related effects on developmental parameters including pre and post-implantation loss, litter sizes, mean viable and nonviable fetuses, fetal sex and weight, external and visceral anomalies. The percentage of fetuses/litter with unossified caudal arches with cartilage present in F_1 Cohort 1a fetuses was significantly increased in the high-dose group, but was not considered adverse due to the lack of increase in a closely related variation at the same ossification site and no changes at multiple other ossification sites.

The test substance showed no potential interaction with the estrogen or androgen pathways evidenced by no effects on sexual maturity, reproduction parameters, reproductive organs, or histopathology.

There was no consistent pattern of effects on thyroid parameters. Mid- and high-dose males of F_1 Cohort 1b had decreased T3 with no corresponding changes in T4 or TSH. Changes in T4 and TSH lacked a dose-response relationship, consistent responses at similar life stages, and consistent correlation with histopathological findings. Thyroid histopathological alterations, comprised of minimal to slight follicular hypertrophy, were seen in 2/29 and 3/23 P females in the mid- and high-dose groups, respectively. There was no correlation with thyroid hormone levels and similar findings were not observed in F_1 Cohort 1b females. There were no consistent correlations between thyroid gland semi-quantitative microscopic scoring and hormone data. Exposure to thyroid modulators typically results in decreased T4, increased TSH, increased follicle height, and follicular cell hypertrophy or hyperplasia which was not observed.

There were no treatment-related effects on developmental neurotoxicity including clinical signs, ophthalmology, FOB, motor and locomotor activity, auditory startle parameters, brain weights, gross brain measurements, microscopic brain measurements and brain neuropathology, and other neuropathological findings.

Based on the results of this study, the overall NOAEL was considered to be 1000 ppm based on the decreased in body weights of F_1 animals. There were no adverse effects on other parameters related to systemic, reproductive, developmental neurotoxicity, and developmental toxicity and endocrine disruption.

B. REVIEWER COMMENTS: The reviewer agreed with many of the study author's conclusions for this study, but differed on several points, including interpretation of the thyroid effects and determination of the parental and offspring NOAEL and LOAEL, as discussed below. The reviewer notes that there is some uncertainty regarding interpretation of

the thyroid hormone assay data in the absence of full SOP information for the thyroid hormone assays, the conditions of animal handling prior to and during blood collection, and blood sampling methods. In addition, thyroid gland weights were measured in g and not mg, which may affect the accuracy of the measurements. Therefore, to be protective of potential thyroid effects to offspring, a conservative approach has been taken in the interpretation of the data.

Parental toxicity (P and F_1 adults): The reviewer notes that no treatment-related effects were observed on P and F_1 parental mortality, clinical signs, body weight, body weight gain, food consumption, hematology, clinical chemistry, testosterone in males, urinalysis and macroscopic findings. In P males at 2000 ppm, statistically significant decreases in monocytes (% and abs, 29% and 33% below controls, respectively) were observed but were within historical control range. In F_1 Cohort 1b parental females at 2000 ppm, statistically significantly increased white blood cell counts (40%), absolute segmented neutrophil (35%) and absolute segmented neutrophils were observed. These findings were not considered treatment-related, based on values being within historical control range, or values in control and/or all treated groups being outside of historical control range. At 2000 ppm significantly decreased triglyceride levels were seen in P females (54%) and in F_1 females at 1000 and 2000 ppm (40% and 49%, respectively), an effect which is not considered to be adverse.

Thyroid effects at 1000 and 2000 ppm were considered treatment related, although consistent changes in P and F_1 adults were not observed. P males (PND 85) did not show effects on thyroid except for a 19% decrease in T4 (not significant) at 2000 ppm. In P females (LD 22), increases at all dose levels in T4 (27%, 34%, 18%, $p<0.05$, 250 and 1000 ppm) and TSH (64%, 164%, 92%; significant only at 1000 ppm) were observed, along with minimal-slight thyroid follicular cell hypertrophy in 2/29 and 3/23 females at 1000 and 2000 ppm, respectively. Semi-quantitative microscopic analysis of thyroid follicles showed significantly decreased colloid area and increased thyroid follicular cell height at 1000 and 2000 ppm. There were no effects on thyroid weights of P animals. In adult F_1 offspring, Cohort 1a (GD 20) females showed no effects on hormone levels or histopathology, and males (PND 148) showed no histopathology changes (hormones not evaluated). F1 Cohort 1b males (PND 175) showed a significant decrease in T3 (19% and 18%) at 1000 and 2000 ppm, but no other effects on hormones or histopathology. F_1 Cohort 1b females (LD 22) showed no effects on thyroid hormones, but at 2000 ppm, significant increases in absolute/relative thyroid weight (18%/25%) were observed. Decreased colloid area/increased follicular cell height were also observed at 2000 ppm. **The parental toxicity LOAEL is 1000 ppm (53.2 mg/kg bw/day in males, 67.6 mg/kg bw/day in females), based on alterations in thyroid hormones and thyroid histopathology in P females during lactation. The parental toxicity NOAEL is 250 ppm (13.9 mg/kg bw/day in males, 16.2 mg/kg bw/day in females).**

Reproductive toxicity: In agreement with the study author, the reviewer notes that there were no apparent treatment-related effects on reproductive indices, precoital intervals, gestation length, estrous cycle, ovarian follicle count, sperm maturation, parturition, lactation, or tissues and organs of the reproductive system. **Therefore, the reproductive LOAEL is 2000 ppm (106.7 mg/kg bw/day in males, 136.8 mg/kg bw/day in females). The reproductive LOAEL is not established.**

Developmental (prenatal) toxicity: In agreement with the study author, there were no treatment-related effects on live litter size, fetal anomalies (external and visceral) fetal weight,

and fetal and pup sex ratio. A significant increase in the percentage of affected fetuses/litter with unossified caudal arches with cartilage present was observed in the mid- and high-dose groups. This skeletal variation without cartilage present was observed in almost all fetuses and litters in all dose groups. Since the two variations are not considered to be independent of each other the increased incidence with cartilage is not treatment-related. **The developmental (prenatal) NOAEL is 2000 ppm (106.7 mg/kg bw/day in males, 136.8 mg/kg bw/day in females). The developmental LOAEL is not established.**

Offspring (postnatal) toxicity: There were no treatment-related effects on live litter size or pup viability, anogenital distance, developmental landmarks, food consumption, organ weights, or histopathology of the F_1 and F_2 offspring. A slight increase in dead fetuses observed at 2000 ppm was due largely to complete litter loss in one litter of 10 fetuses and was considered an incidental finding. F_1 males showed transient decreases in mean body weight beginning in the latter part of lactation. Body weight gain of F_1 male pups of the 2000 ppm group was significantly decreased from PND 4-18 by 10%, correlating with absolute mean body weights decreases of 6-7% (n.s.) from PND 14-21. After weaning, body weights of F_1 male offspring remained decreased from PND 24-28 by 7-10% (s.s.), but thereafter weights were comparable to controls. The decreased body weights during late lactation and early post-weaning days may reflect toxicity from increased test material intake resulting from increased food consumption of the dams during this time (test diet concentrations were not adjusted during lactation or post-weaning), along with the onset of solid food consumption by the pups. Mean body weights of F_2 females at 2000 ppm were also significantly decreased by 8-11% on PND 24-28 but not at later times. Although the decreases in pup weights were transient, they were considered adverse, due to the young age of the animals.

Changes in thyroid hormone levels, colloid area and follicular cell height were seen in offspring but were not consistent across cohorts or life stages. In F_1 PND 4 offspring, decreased T4 was observed at 1000 and 2000 ppm (24% and 35%; significant at high dose) but TSH was unaffected (histopathology was not evaluated). At PND 23, male and female F_1 pups (cohort 3) at 2000 ppm showed statistically significant increases in T4 (53%/22%), significant increases in TSH in females (21%/41%) and significantly decreased colloid area/increased follicular cell height in both sexes. In contrast, F_2 pups at PND 23 showed only nonsignificant increases in T4 in males (20%) and TSH in females (26%). PND 45 females at 2000 ppm showed increased T4 (52%, $p<0.05$), TSH (41%, n.s) and significantly altered colloid area and follicular cell height (-19%/+60%). Follicular cell height and T4 or TSH levels did not show a consistent positive correlation in treated animals. No effects on thyroid weight or microscopically visible thyroid follicular cell hypertrophy were seen in young offspring. No changes in thyroid parameters were observed in any offspring at 250 ppm. **The offspring toxicity LOAEL is 1000 ppm (53.2 mg/kg bw/day in males, 67.6 mg/kg bw/day in females), based on thyroid hormone and microscopic thyroid alterations in F_1 PND 23 male pups and decreased T4 in F_1 PND 4 pups (blood pooled by litter). The offspring toxicity NOAEL is 250 ppm (13.9 mg/kg bw/day in males, 16.2 mg/kg bw/day in females).**

Thyroid toxicity (parental and offspring): Details of thyroid observations in P and offspring animals at various life stages, and identification of parental and offspring NOAELs and LOAELs, were summarized above. Although the reviewer agreed with the investigators that the pattern of thyroid changes was not consistent in the different life stages, differences in thyroid effects may also reflect differences in susceptibility of specific life stages. Due to lack

of details on the blood sample collection conditions and SOPs for conduct of the thyroid hormone assays, a conservative approach was taken in the interpretation of the results. The combination of alterations in blood thyroid hormone levels and semi-quantitative morphometric changes were considered to be evidence of perturbation of thyroid homeostatis and considered to be treatment-related. Based on the findings of this study, offspring thyroid changes were not seen at lower dose levels than adults and there does not appear to be evidence of increased offspring susceptibility with respect to alterations in thyroid function.

Developmental neurotoxicity: In agreement with the conclusions of the study author, the reviewer notes that, under the conditions of this study, there was no effect of treatment on clinical signs, ophthalmology, FOB, motor and locomotor activity, auditory startle parameters, brain weights, gross brain measurements, microscopic brain measurements and brain neuropathology, and other neuropathological findings. **The developmental neurotoxicity NOAEL is 2000 ppm (106.7 mg/kg bw/day in males, 136.8 mg/kg bw/day in females). The developmental neurotoxicity LOAEL is not established.**

This study is classified as **Acceptable / Non-Guideline** and does not fully satisfy the guideline requirement for an extended one-generation reproductive toxicity study (OECD 443) in the rat. The guideline was modified to test the potential reproductive, developmental neurotoxicity, and thyroid effects of the test substance and is adequate to assess these parameters. The OECD guideline recommends that Cohort 3 animals be used for immunotoxicity evaluations, but immunotoxicity tests were not conducted. However, the study, as conducted, does not indicate effects on the immune system based on hematological, organ weight or pathological observations.

C. STUDY DEFICIENCIES: The following deficiencies were noted:

The author did not provide adequate descriptions of the procedures used for the FOB testing including the size of the open field, duration of the observation period in the open field, procedures used to determine landing foot splay or scoring criteria. The study report did not include a list of the FOB scoring criteria but did cite references for standard procedures for these assessments.

Blood clotting time/potential was not measured.

Immunotoxicity evaluations were not conducted and an explanation for not conducting the tests was not reported. However, the study report notes that the current study was based on the principles of a modified OECD extended one-generation reproductive toxicity test guideline.

Detailed SOPs for thyroid assay methodology were not available for review.

Thyroid gland weights were measured in grams instead of milligrams, reducing the sensitivity of the measurements.